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THE OXIDATION OF SULFUR BY MICROÖRGANISMS IN ITS RELATION TO THE AVAILABILITY OF PHOSPHATES[1]

HARRY C. McLEAN

New Jersey Agricultural Experiment Station

Received for publication September 28, 1917

INTRODUCTION

It has already been shown in previous experiments carried out at the New Jersey Agricultural Experiment Station (44, 45, 46, 47,) that:

1. The oxidation of sulfur in sand and soils was found to render soluble in neutral ammonium citrate and water appreciable quantities of the phosphorus of raw rock phosphate.

2. The largest amount of available phosphoric acid at the end of 30 weeks was found in composts made up with a red silt loam soil. In this case 85 per cent of the phosphorus in the floats had been rendered available.

3. In both sand and soils, the oxidation of sulfur as measured in terms of sulfates, paralleled the increase of available phosphoric acid.

4. Moisture was an influential factor in the oxidation of sulfur and the making available of raw rock phosphate.

5. Vegetation experiments showed that the sulfur-floats-soil compost could be employed to advantage as a substitute for acid phosphate.

6. Experiments carried out under field conditions corroborated the results obtained in the laboratory.

In view of the above findings, it was deemed advisable to start a number of new experiments designed to involve numerous attending factors and influences. Therefore, since the above investigations were reported there has been an accumulation of valuable data, the importance of which not only surpassed expectations but predictions as well. The great value of inoculation was established and aeration shown to be a vital factor. The purpose of this paper is to set forth the results of these investigations.

Before entering upon a discussion of the results obtained it seems desirable to refer to certain publications on sulfur oxidation, in part, reviewed in an earlier paper (47), which have a more or less direct bearing on the problem at hand. The numerous investigations which have been carried out by mixing

[1] Portion of a thesis submitted to the faculty of Rutgers College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, September, 1917 (Ref. no. 50).

The writer is indebted to Dr. J. G. Lipman for originally suggesting the problem and for valuable advice rendered during the progress of the work.

floats with decaying organic matter, etc., in an endeavor to render insoluble phosphates soluble, have been reviewed elsewhere (47, 49, 50). In this place it need be stated merely that the results obtained in such experiments were unsatisfactory.

HISTORICAL

The fact that elemental sulfur is transformed to sulfates in soils was recorded in 1869 by Marés (53). In answer to the question as to what becomes of sulfur which is applied to soils, he says: "It is transformed into sulfuric acid which combines with the bases present." In order to demonstrate this he made a chemical examination of a calcareous soil which had received annual additions since 1858 of elemental sulfur and also soil from the same field which had not received any sulfur. He found large amounts of water-soluble sulfates in the former soil but none in the latter. The author emphasized the importance of having the sulfur evenly distributed in the soil, and of having it in a finely divided state, if it is to be oxidized rapidly. He also noted that the transformation of sulfur into sulfates was more rapid in soils which had been fertilized. In experiments designed to determine whether hydrogen sulfide is an intermediate product in the oxidation of sulfur, he found that such was not the case. Evidently he did not know that sulfur oxidation is largely a biological process, as he states that "the surface soil is a porous layer which has a very strong oxidizing power." He concluded that: (1) Sulfur may be transformed into sulfates by direct oxidation or else hydrogen sulfide might be an intermediate product, which would be converted into sulfates as soon as formed, or under favorable conditions it would be converted into sulfuric acid which would in turn act on any bases present in the soil; and (2) in certain soils, potassium might be brought into a soluble form by the oxidation of the sulfur.

In 1877 it was suggested by Charles F. Panknin, of Charleston, S. C., that sulfur, if mixed with ground bone or ground mineral phosphates, would be oxidized to sulfuric acid when incorporated into cultivated soil. He applied for a patent on his process in April, 1877, and succeeded in August of the same year in securing Letters Patent 193,890. In his application for the patent Panknin made the following statement:

> My invention relates to a compound consisting of a mixture of finely-powdered bone or mineral phosphates and finely-powdered sulfur, the said ingredients being mixed together and applied in a dry state, and the conversion of the insoluble phosphate of lime is effected by the sulfuric acid eliminated by the natural oxidation of the sulfur of the mixture in the soil to which it is applied.
>
> To prepare the fertilizing compound, take 95 parts of the insoluble phosphates, (either bone or mineral phosphate,) in a finely powdered state, and mix with it 5 parts of finely-powdered sulfur, both ingredients being in a dry state, though I do not confine myself to these proportions. This mixture though only mechanical in its character, when applied to the soil, becomes slowly transformed, the sulfur being first converted into sulfuric acid by a natural process of oxidation, and when so converted, uniting with the insoluble phosphates to form a soluble phosphate, which is dissolved by the moisture of the soil, and acts in the same manner as phosphates made by artificial process.

In September, 1904, a patent application was submitted by William B. Chisholm of Charleston, S. C. and Patent 824,280 was secured by him on June 26, 1906. Chisholm makes the following statement:

> I find that the percentage of sulfur, by weight, to give effective results may in some instances be as low as forty pounds to the ton of the mixture—i.e., forty pounds of sulfur to nineteen hundred and sixty pounds of phosphate rock—and in other instances from sixty to one hundred pounds of sulfur to the ton of mixture. In the practical use of my invention I find that the crops produced are apparently far superior to what might reasonably be expected from the amount of available phosphate, nitrogen, and potash or other ingredients present in the final mixture. This may be due in part to the extreme fineness and intimate association of the admixed particles tending to facilitate their ready and uniform distribution through the soil, their chemical interaction under the influence of the moisture of the soil and the infiltrations of rain, and their increased solubility in said condition of impalpable powder; but I am further led to believe that the sulfur itself has an independent effect as a germicide, destroying bacterial and insect life prejudicial to the plant and either not interfering with or positively stimulating helpful bacteria.

The above citations from the Panknin and Chisholm patents bring out some very interesting facts. It is evident that Panknin recognized nearly forty years ago the value of sulfur oxidation for transforming insoluble into soluble phosphates. He did not know, of course, that the process of sulfur oxidation is largely biological in character. Neither did he recognize that the very small quantities of sulfur recommended by him, namely 1 part to 20 parts of phosphate, were entirely inadequate for accomplishing the desired reaction. The same criticism may be made of the process proposed by Chisholm. In both instances relatively small amounts of sulfur are directly applied to the soil with the expectation that the sulfuric acid ultimately produced would attack the insoluble phosphates.

In attempting to determine the value of sulfur as a fertilizer, Degrully (19) discovered in 1911, that the sulfur applied at the rate of 109 gm. per square meter was largely oxidized to sulfates.

Boullanger (7), and later Boullanger and Dugardin (10), were able to prove a favorable effect of sulfur on crop yields. Since the corresponding results in sterilized soil were not marked, they concluded that the oxidation of sulfur is brought about by bacterial activities. The results just noted were confirmed by Demolon (21, 22) in 1912 and again in 1913 (23).

In his earlier experiments he employed 5 gm. of sulfur to each 100-gm. portion of soil. Notwithstanding the relatively large application, much of the sulfur was oxidized to sulfuric acid. In other experiments he added 1 per cent elemental sulfur to a sterilized and an unsterilized soil. He found very little of the sulfur to have been oxidized in the sterilized soil at the end of two months, although a considerable amount was oxidized in the unsterilized soil. He obtained no reaction for gaseous SO_2 or H_2S in this experiment. The author thinks that sulfur added to soils may act as a source of sulfuric acid. Subsequently, Demolon (23) in another paper records experiments in which he added 1 per cent of sulfur to garden soil, kept the mixture moist and at a

temperature of 20°C. Under these conditions appreciable quantities of sulfuric acid were formed, as shown by the amounts of calcium sulfate found in the mixture at the end of 40 days. It was observed at the same time that the oxidation of the sulfur was most active in a non-sterilized soil.

In vegetation experiments it was found that when sulfur was supplied in an amount equal to that of the nitrogen, the yields were as good as those obtained from a complete fertilizer. He ascribed the beneficial action of sulfur as having been due to its influence on the activities of soil bacteria, as well as to its gradual transformation into sulfuric acid, and the reaction of the latter with basic material to form an increased supply of mineral plant-food.

The oxidation of sulfur was made the subject of study also by Brioux and Guerbet (11). They dealt particularly with the influence exerted on the oxidation of sulfur by the soil itself and by certain carbohydrates, peptone and certain other nitrogenous materials. Their investigations showed that carbohydrates had a retarding effect, while peptone accelerated it so that in 30 days 82 per cent of the 4 gm. of the flowers of sulfur added, was oxidized to sulfates. In sterile soil and in soil which had been sterilized and inoculated with soil infusion, they found 1.6 per cent and 94.2 per cent, respectively, of the 0.20 gm. of precipitated sulfur originally added, to be oxidized to sulfates in 16 days. Calcium carbonate was also found to have enhanced the rate of sulfur oxidation. Some bacteria which would oxidize sulfur were isolated, one of which would oxidize 18.6 per cent as against 3.6 per cent found in the sterile check. They concluded that the oxidation of sulfur in soils is a very complicated process which involves the activities of a number of species of bacteria. They think that perhaps the transformation is brought about in a way less direct than pure oxidation and in certain cases with the formation of hydrogen sulfide as an intermediate product.

In another experiment they found the oxidation of sulfur in soil to have exerted a solvent action on the soil minerals. By extracting the soil with distilled water they obtained a considerable increase of potassium and calcium over that obtained from the check soil. A test showed that none of the soil phosphorus had been rendered soluble.

In 1912 von Feilitzen (27) carried on some pot experiments designed to study the fertilizing action of flowers of sulfur on horse-beans and ray-grass. After harvesting the crops he tested the various soils for water-soluble sulfates and acidity. Very little difference was noted in the sulfate content of the soils, although a litmus test showed the acidity to have been greatly increased by the sulfur.

Pfeiffer and Blanck (57) during the same year found applications of sulfur at the rate of 267 and 524 pounds per acre, on field plots, to have increased the sulfate content of the soils. This increase was directly proportional to the amount of sulfur applied. The check plot was found to be neutral in reaction, while the others were strongly acid, showing that the sulfur had been oxidized to sulfuric acid.

Some interesting data concerning the influence of sulfur on soil acidity are recorded by Lint (40). In order to ascertain the rate of oxidation of sulfur in soil, 100-gm. portions of it were treated with 33 mgm. of flowers of sulfur, which is an addition equivalent to 100 pounds of sulfur per acre-foot of soil. The moisture content was 20 per cent. The acidity was found to increase up to the eighth week when all the sulfur was apparently oxidized. Experiments with a heavy clay loam and a sandy loam soil showed a much more rapid oxidation of the sulfur in the latter soil. Soils with a water content of 20 per cent when allowed to dry did not cause as rapid an oxidation as those maintained at an optimum moisture content throughout the experiment.

Somewhat later Pitz (59) found elemental sulfur to increase soil acidity, in amount proportionate to the per cent of sulfur applied, thus corroborating the work of other investigators. He also found the number of bacteria which would grow on agar plates to decrease after a certain period in a silt loam soil which had received an application of sulfur.

Experiments reported a short time later by Duley (25) confirmed the results just noted. He, however, found the sulfur to be oxidized to sulfates in both quartz sand and soil. The lime requirement of the soil which had been treated with sulfur was directly correlated with the amount of soluble sulfates formed.

Shedd (65) also studied the oxidation of sulfur in soil and sand. With soil to which sulfur was added in amounts varying from 100 pounds to 7000 pounds per acre, he found by analysis, that 60 to 80 per cent of the sulfur, regardless of the amount added, was oxidized after a period of about 4 months. Sulfur was oxidized also in the sand but not to the extent that it was in the soil.

Kappen and Quensell (35) made a study of the sulfur cycle in soils. In connection with these experiments they studied the oxidation of ground rhombic sulfur, colloidal sulfur and the so-called "Schwefel milch." The latter was prepared by mixing together 2 parts of sulfur, 1 part of calcium hydroxide and 13 parts of water. The solution was heated until the constitutents dissolved. After diluting and precipitating with dilute hydrochloric acid, the precipitated sulfur was filtered and washed. Two hundred milligrams of the dry sulfur thus prepared and of the ground rhombic sulfur were mixed with the different soils under investigation, incubated for 6 weeks, extracted with 10 per cent hydrochloric acid and the sulfates determined in the extract. The results which they obtained are quoted below to show the striking oxidation of the precipitated sulfur.

SOIL	SO_4 FROM 200 MGM. OF SULFUR	
	Rhombic sulfur	Precipitated sulfur
	mgm.	*mgm.*
Glass sand	3.69	44.63
Sandy soil	3.81	144.22
Loam soil	16.44	227.57
Meadow soil	10.91	284.01

Brown and Kellogg (13) report some experiments on the so-called sulfofying power of soils. They find that each soil has a definite sulfofying power. Organic matter was found to increase the amount of sulfur oxidized. Manure and green manure increased it up to a certain point. Manure was found to play an important part in the oxidation processes, and the optimum moisture condition was apparently 50 per cent of complete saturation. Other experiments which they carried out show that aeration is an important factor, as is also the absence of carbohydrates. The presence of bacteria is essential, although the production of a small amount of sulfates was brought about by chemical action.

Brown and Johnson (12) made a study of the effect of gypsum, acid phosphate, rock phosphate, alone and with gypsum, and mono-calcium phosphate on sulfur oxidation in an alkaline soil high in organic matter. They found all of the above materials to exert a stimulating action when applied in amounts based on actual field conditions. The rock phosphate, mono-calcium phosphate and gypsum exerted the greatest stimulating action. They report other experiments in which calcium and magnesium carbonates were employed. With small applications of calcium carbonate they obtained greatly increased sulfur oxidation, and even with excessive amounts a favorable effect was obtained. On the other hand, small applications of magnesium carbonate increased sulfofication, while large amounts exerted a depressing action.

Ames and Boltz (1), in their recent study of the effect of sulfur on the growth of plants, found that it produced increased yields when applied to certain crops. In order to determine whether or not this beneficial action was due to the solvent action on the mineral soil constituents of the acids produced during the sulfofying processes, they conducted a composting experiment in which sterilized and unsterilized soils, a loam and a clay soil, and pure quartz sand were used. To 500-gm. portions of the soil and sand were added 0.25 gm. of flowers of sulfur and Tennessese phosphate rock in amounts containing 0.06 gm. of phosphorus. To each mixture was added 400 cc. of distilled water. After two months they found but a slight increase in the availability of the phosphorus, as measured by 0.2 per cent hydrochloric acid. The acidity of the mixture had also increased but little.

Among the more recent investigations which have been made relating to sulfur oxidation, should be mentioned those of Ames and Richmond, and Brown and Warner.

Ames and Richmond (2) report experiments in which the effect of sulfur, gypsum, and acid phosphate on the changes in nitrogen and phosphorus of horse manure were studied. They found all of these materials to be very effective in reducing the loss of dry matter in manure; sulfur flowers being the most effective. The materials also reduced the loss of nitrogen from the manure. Manure treated with acid phosphate, sulfur, and calcium phosphate lost, on an average, 3.5 per cent of its total nitrogen in 250 days, as compared with a loss of 10.5 per cent from untreated manure. A study of the hydrogen

sulfide evolved from the different manure mixtures showed the gas to be given off in largest amounts from the ones treated; the largest being evolved from those treated with sulfur.

The acidity of water extracts from the mixtures after fermentation showed that the sulfur-treated manure had increased in acidity, while the untreated manure had become alkaline in reaction.

The manure treated with flowers of sulfur produced water-soluble sulfates equivalent to 23.4 gm. of sulfuric acid, as compared with a loss of about 4 gm. of sulfuric acid from the untreated manure.

Brown and Warner (14) report experiments which confirm the results reported previously by the writer and also those reported in this paper. They made up mixtures of sulfur and floats, with compost, horse manure or cow manure. These mixtures were allowed to ferment for varying lengths of time and there was found to be an enormous increase in the production of available phosphorus which continued up to fifteen weeks. The increase in available phosphorus was greater where the sulfur and floats were intimately mixed with the manure than where they were added to the manure in layers. All of the manures and the soil tested contained efficient sulfofying organisms. The authors think that "further tests of this method of producing available phosphorus may prove it to be of considerable practical value."

In view of the fact that considerable work has been carried on in which sulfur has been used in agricultural practice, the author thought that it would be advisable to give a brief résumé of at least some of the more important results obtained from the use of sulfur as a fertilizer, in both this country and in Europe; especially so since a majority of the experiments have a direct relation to the question of sulfur oxidation. From a study of them we are able to obtain some indication as to the conditions, both physical and chemical, under which the most efficient oxidation of sulfur can be obtained in soil.

SULFUR IN ITS RELATION TO PLANT GROWTH

Up to the present time elementary sulfur or its compounds have not been generally considered essential as a constituent of commercial fertilizers. Hence their use as a fertilizer has been confined almost entirely to experiments. In recent years investigations on the effect of sulfur on plant growth have been quite common. Numerous reports have been made public from time to time. Possibly this stimulus to sulfur research has been due to the conflicting nature of these reports; for some investigators record a beneficial effect of sulfur on the growth of plants, others, a detrimental effect, and still others, no effect whatever. Even more conflicting are the explanations offered as to the action of sulfur in soils.

However, it is generally agreed that plants contain, and require, sulfur, especially those rich in albuminoids. Even with the imperfect analyses of former years it was found that plants such as cabbage and alfalfa had a high

sulfur content. By an improved method of analysis Hart and Peterson (31) succeeded in demonstrating a still higher sulfur content in these and other crops. Among others Shedd (65) and Peterson (55, 56) report considerable amounts of sulfur in plants. Hart and Peterson (31) also showed that: "Normal soils are relatively poor in sulfur trioxide. An acre foot will contain from 1000 to 3000 pounds of sulfur trioxide. Soils cropped from fifty to sixty years and either unmanured or receiving but slight applications during that period have lost on the average 40 per cent of the sulfur trioxide originally present, as determined by comparison with virgin soils." These results were later confirmed by Shedd (64) who made a study of the sulfur content of Kentucky soils. Also Kossovich (36) concluded that an impoverishment of the soil as to sulfates is possible and that sulfate must be added in fertilizers. Subsequent work of Robinson (62) showed that most of the important American soils were low in sulfur. Thus an appreciable presence of sulfur in plants with a possible appreciable absence of it in soils would seem to justify the investigations that have been and are being made.

Most of these investigations indicate beneficial effects from the use of sulfates. The methods used and plants under trial have varied. Among the different sulfur compounds the sulfates were the first observed to enhance crop yields. Bogdanov (5) in 1899 found a response to fertilizers containing sulfates. In 1905 Dymond, Hughes and Jupe (26) demonstrated the value of sulfates, particularly with crops containing a high proportion of albuminoids. Lierke (38) reports that fruits responded to fertilizers containing considerable amounts of sulfates. Reimer (61), Hart and Tottingham (32) and many others also record beneficial results from the use of sulfates.

Other compounds of sulfur have also been tried. Pyrites at the rate of 89.2 to 178.5 pounds per acre were found by Vermorel and Danthony (73) to augment the yields of wheat and beans 30 to 60 per cent when used in combination with organic matter. Galtsev and Yakushkin (29) likewise found pyrites to exert a beneficial effect. Sulfuric acid tested by Rabaté (60) and by Demolon (23), sulfurous acid and carbon bisulfid tried by Demolon (23) and various sulfites employed by others were found to exert a fertilizing effect upon plants.

Quite recently there has developed a wide interest in elementary sulfur, although its fertilizing effect on vines was noted over half a century ago. In 1858 Marés (42) discussing some of his own observations, said: "On peut considerer le soufre, pour les vignes, comme un engrais, on plutot comme un amendement d'un ordre particulier."

From time to time the belief had also been expressed that sulfur where used for potato diseases was not only effective in reducing the disease but also in increasing the yield. In 1911 Chancrin and Desriot (16), using 223 to 446 pounds of sulfur per acre, noted an increase in potato and beet crops. In 1912 Chancrin and Desriot (17) again report increased yields. A continuation of this work was reported in 1913 by Desriot (24). Boullanger (8) tested flowers of sulfur in comparison with various sulfates on carrots, beans,

celery, spinach, lettuce, sorrel, chicory, onions, and potatoes. He reports in 1912 that in most cases increased yields were obtained, but that flowers of sulfur were especially favorable. In 1913 Boullanger (9) reports experiments which show the importance of sulfur as a fertilizer. Favorable results are also reported by Vercier (71), Liechti (37), and by Demolon (21, 22). Demolon (23), continuing his work, stated a year later that "potatoes were especially aided by applications of sulfur." Good results were secured by Urban (70) in the growing of sugar beets; by von Feilitzen (27) on potatoes; by Bernhard (4) on hoed crops, especially potatoes and mangolds; by Magnien (51) on turnips and beets; by Tottingham (68) on rape and radishes; by Reimer (61) on alfalfa; and by Ames and Boltz (1) on clover. Shedd (65) of the Kentucky Experiment Station, using sulfur and various compounds of sulfur as fertilizer for soybeans in pot experiments, concluded that the best results were obtained with elementary sulfur. Duley (25) found sulfur to increase markedly the yield of clover, as well as the production of nodules. He also found sulfur to increase chlorophyll production in the case of corn.

While sulfur was thus found to be beneficial the extent of the benefit very often varied. It came under the observation of some that the effect was modified by the presence or absence of organic matter. In pot experiments where sulfur was applied at the rate of 44.6 to 89.2 pounds per acre, Vermorel and Danthony (73) noted increased yields only where organic matter was added. They stated that sulfur was without effect when used on soils lacking in organic matter. Chauzit (18), in 1914, in tests with grapes, obtained the best results where sulfur was used in conjunction with a fertilizer high in organic matter. Vermorel (72) in the same year cites experiments which show that an application of manure enhanced the benefits of sulfur. Zolla (76) asserts that sulfur has a marked effect as a fertilizer when the soil is well stocked with organic matter; and that the effect of sulfur decreases as the content of organic matter is decreased. Sherbakoff (66) found the injurious after-effects of sulfur to be greatest on parts of the field poorest in humus. In accord with these findings are the observations of Sabashnikov (63) and others that sulfur does best when applied to a fertile soil.

A further modification of the effect of sulfur was found to depend upon its use in combination with fertilizers. For instance, Gianitto (30) obtained an increase in yield of potatoes when 400 pounds of sulfur per acre were applied with other fertilizer, and a decrease where the sulfur was applied alone. Chauzit (18) reports in 1914 that when 178 pounds of sulfur were used without the addition of other fertilizers, the yield of grapes was increased 2 to 20 per cent; when used with other fertilizers it was increased from 9 to 35 per cent. Similar results were noted by McConnel (48), Janicaud (34), and others.

At times the extent of the benefit from sulfur has been so small as to be negligible. In fact, its application has frequently been described as producing no results whatever. Vermorel and Danthony (73) claimed that sulfur showed no effect when used on soils lacking in organic matter and receiving

nitrogen in the form of nitrate of soda. Shedd (65) announces that in some of his experiments there was no effect produced by certain forms of sulfur. Tritschler (69) obtained but slightly increased yields of mangelwurzels. Voelcker (74) found sulfur to be without effect on mustard, rape and clover. Thalau (67) and many others term their results as "inconclusive." Bosinelli (6) thinks that the commercial use of sulfur as a fertilizer is of very doubtful value. Pfeiffer and Blanck (57) found that applications of sulfur at the rate of 267 and 524 pounds per acre did not increase the yield of oats; in fact, in many cases the opposite result was obtained. Pfeiffer and Simmermacher (58) obtained the same results a year later with beets. They concluded that the general use of sulfur as a fertilizer is not justified.

Furthermore, in the experiments of some investigators sulfur has not only failed to show a benefit, but has passed from a neutral into an injurious substance in its effect upon plant growth.

Demolon (23) in 1913 noted that cereals were injured by applications of sulfur on light soils poor in calcium carbonate. Gianitto (30), it will be recalled, noted a decreased yield of potatoes where sulfur was used alone at the rate of 400 pounds per acre. Deleterious results were likewise obtained by Janicaud (34) whenever sulfur was used alone. Although Hart and Tottingham (32) secured good results with sulfate on red clover, they found elemental sulfur to exert a harmful effect, this being attributed to incomplete oxidation of the sulfur, or to acidity resulting from the formation of sulfuric acid.

Usually the harmful effects of sulfur have been noted on the subsequent crops. It was reported by Wheeler, Hartwell and Moore (75) in 1899 that unless a considerable quantity of lime were added, injury befell cereals following potatoes which had received an application of sulfur for the prevention of potato scab. This injurious effect they ascribed to free acid in the soil formed from the slow oxidation of the sulfur. Regarding the effect of sulfur treatment on succeeding crops, Sherbakoff (66) states that where it was applied at the rate of 900 pounds per acre, there was a noticeably poor stand of clover, but where it was applied at this rate in combination with lime and additional fertilizer, the injurious effect was less marked. Also von Feilitzen (27) and Lint (41) report similar results. Lint states that in two instances where sulfur was applied in the row to potatoes, hay failed to grow in the following year—thus showing prominent bare streaks across the field where the rows had been. The evidence of this injury slowly disappeared, and by the end of summer no traces of injury remained. A year later Lint (42) reports that no appreciable injury was noticed on a succeeding crop of alfalfa where lime was applied to the soil.

Since it appears that sulfur and its compounds usually exert an influence upon the higher plants it would be natural to expect an influence, also, upon the lower forms of plant life—the microörganisms of the soil. R. Lieske (39) observes that in certain cases bacteria cause denitrification as well as sulfur oxidation. Brioux and Guerbet (11) infer that sulfur incites a complicated

bacterial process to effect its oxidation. That sulfur exerts a stimulating action on the bacteria which break down the nitrogenous matter of the soil to ammonia is the belief of some scientists; among whom are Boullanger and Dugardin (10), also Bosinelli (6). Sulfates, however were found by Fred and Hart (28) to produce no effect upon the rate of ammonification or the destruction of organic matter in the soil, and to cause no appreciable increase in the number of bacteria which would grow on agar plates. Pitz (59) obtained the same results. In the use of elemental sulfur Pitz records a decrease in the total number of bacteria but an increase in ammonification, accompanied by a parallel decrease in nitrate formation. Although there has been considerable work done by other investigators the relations in question are not well understood—beyond that the action on the soil flora, brought about by sulfur, is certainly a factor to be considered in any theory as to the effect of sulfur upon higher plants.

The rôle that sulfur plays in the soil—the theory of its action—is an undecided question. Various views have been expressed. It has been suggested by Bernhard (3), Chancrin and Desriot (16), Boullanger (8) and Heinze (33), that it functions as a disinfectant or partial sterilizer. To quote the opinion of Chancrin and Desriot: "The beneficial effects of sulfur may be due to action similar to that of partial sterilization by heat, carbon-bisulfid, toluene, etc." The belief that it has a physical effect upon the soil is maintained by Bernhard (3) and Degrully (20). A catalytic function is ascribed to it by Cettolini (15) and Boullanger (8) and others. Still other theories have been advanced by Miége (54) and many others. Worthy of mention, perhaps, is that of Demolon (23) who attributes the fertilizing effect of sulfur to its progressive transformation into sulfuric acid, thereby supplying sulfur to the plant and rendering soluble other mineral constituents of the soil. Still more worthy of mention is the suggestion recently made by Lipman (43), that the change of sulfur to sulfuric acid in the soil may be employed for converting sodium carbonate into sodium sulfate and the making productive the barren alkali areas.

In a brief review of the numerous papers on the relation of sulfur to plants it is quite necessary to omit details, or even to avoid mention of some experiments. Nevertheless, the striking importance of the subject cannot be ignored. The sulfur requirements of plants, the possible absence of it in soils and the mode or material for its application as a fertilizer are important questions to consider. Of no less interest to the scientific man are its influence on plant growth, upon microörganisms, and the theories of its action. It is safe to assume that the future will not lack in enlightening investigations upon sulfur in its relation to plant growth.

SERIES 1. FURTHER STUDIES ON ECONOMICAL SULFUR-FLOATS COMBINATIONS

Since the experiments already reported (47) dealt with relatively short incubation periods and yielded data that were somewhat conflicting, it was deemed advisable to try a similar experiment for a longer period of 24 weeks.

In this instance, however, greenhouse soil was used instead of Sassafras loam soil and all the composts were inoculated with infusion; the composts being made in the same manner. The plan of the experiment, and the ammonium-citrate-soluble P_2O_5 found in the composts at the end of 24 weeks[2] are recorded in table 1.

In considering the extent of the increase we observe that there is a consistent increase as the amount of sulfur and floats becomes larger. There is exhibited the wide variation of 107.44 mgm. with 0.6 gm. of sulfur and 2 gm. of floats to 1977.55 mgm. of available P_2O_5 with 9 gm. of sulfur and 30 gm. of floats. The point of maximum concentration was evidently not reached

TABLE 1

Influence of varying proportions of sulfur and floats on the accumulation of available phosphoric acid

LABORATORY NUMBER	ADDITIONS	AMMONIUM-CITRATE-SOLUBLE P_2O_5		
		Average	Increase over check	Increase per gram of floats
		mgm.	*mgm.*	*mgm.*
1	None	101.05		
2	15.00 gm. floats	159.25		
3	0.20 gm. floats. 0.60 gm. sulfur*	216.25	107.44	53.72
4	1.20 gm. sulfur. 4 gm. floats	498.75	382.18	95.55
5	1.80 gm. sulfur, 6 gm. floats	750.00	625.67	104.41
6	2.40 gm. sulfur, 8 gm. floats	877.50	745.51	105.69
7	3.00 gm. sulfur, 10 gm. floats	1075.00	935.15	93.52
8	3.60 gm. sulfur, 12 gm. floats	1141.25	993.64	82.80
9	3.00 gm. sulfur, 3 gm. floats	674.38	561.69	187.23
10	3.00 gm. sulfur, 6 gm. floats	891.38	767.05	127.84
11	3.00 gm. sulfur, 9 gm. floats	1061.25	925.28	102.81
12	3.00 gm. sulfur, 12 gm. floats	1270.00	1122.39	93.53
13	6.00 gm. sulfur, 20 gm. floats	2121.25	1942.60	97.13
14	7.50 gm. sulfur, 25 gm. floats	2099.60	1901.55	76.06
15	9.00 gm. sulfur, 30 gm. floats	2195.00	1977.55	65.92

* Sulfur flour was used in all the experiments reported in this paper.

although it appears to have been nearly attained in the last three composts. In the four cases where the quantity of sulfur remained the same (3 gm.) and the amount of floats increased from 3 to 12 gm., the amount of the phosphoric acid of the floats made available continued to increase, indicating that here, too, a prohibitive concentration point was not reached. From the viewpoint of increase in availability per gram of floats supplied we find, on the contrary, that the unit production diminishes beyond certain points of concentration. Starting with the 0.6 gm. of sulfur and 2 gm. of floats we secure a gradual increase up to 105.69 mgm. with 2.4 gm. of sulfur and 8 gm. of floats, beyond

[2] The methods of analysis used are reported in a previous paper (47).

which point a decrease results. Starting again with the 3–3 sulfur-floats mixture, we obtain the highest production of unit available P_2O_5, namely, 187.23 mgm., which decreases as the amount of floats increases, until with 12 gm. of floats we have but 93.53 mg. of available P_2O_5 per gram of floats. To render less bewildering any selection of the combination which is the most efficient it probably would be helpful to place a cash valuation upon the P_2O_5 made available. This might best be done by a calculation on the basis of a ton of compost; valuing the sulfur, let us say, at 1 cent per pound and the floats at $\frac{1}{2}$ cent per pound. The net amount of available P_2O_5 produced per ton of compost, and the cost per pound of this P_2O_5 which has been made available, are shown in table 2.

TABLE 2

Amount and cost of phosphoric acid rendered available per ton of compost

COMPOST NUMBER	AVAILABLE P_2O_5	COST PER POUND AVAILABLE P_2O_5
	pounds	*cents*
1		
2		
3	2.09	14.91
4	7.26	8.37
5	11.61	7.66
6	13.26	8.74
7	16.55	8.55
8	17.18	9.67
9	10.58	8.02
10	14.06	7.83
11	16.52	8.11
12	19.52	8.00
13	30.82	8.23
14	28.70	10.52
15	28.44	12.14

A study of tables 1 and 2 shows that no direct relation exists between the highest total or the highest unit quantity of available P_2O_5 and the actual cost per pound of the same. Our highest total production of 1977.55 mgm. of available P_2O_5 cost at the rate of 12.15 cents per pound. Our highest unit production of 187.23 mgm. shows a cost of 8.04 cents per pound. But our minimum cost was 7.68 cents, in the case of the mixture containing the 1.8 gm. of sulfur and 6 gm. of floats—a combination which gives a total amount of 625.67 mgm. of P_2O_5 and a unit amount per gram of floats of 104.41 mgm. Nevertheless, it cannot be said that this compost is the most economical, for other things must be considered. For instance where the cost is 7.68 cents per pound only 12.51 pounds of available P_2O_5 are produced. It would require three such composts of 1 ton each to furnish as much P_2O_5 as is made available in compost 13, where the cost was only 8.25 cents per pound. Undoubtedly, the margin of expense is here too small to warrant such an extensive handling.

However, just what ratio of sulfur to floats would be the most economical cannot be unqualifiedly stated, as it would depend largely upon environmental conditions.

It must be remembered that the length of incubation for this experiment was for a period of 24 weeks. The results are obviously much more satisfactory than those obtained in series 3, where a 12-week incubation period was used. Very likely a longer time than 24 weeks and a more efficient sulfofying flora would be more satisfactory, as a larger amount of available P_2O_5 would be formed, which would reduce the cost per pound.

If a conclusion were drawn from the data here presented it would be that a compost made up in the ratio of no. 13, which is at the rate of 120 pounds of sulfur and 400 pounds of floats to 1 ton of soil, would be the most economical. Whether or not further investigations will confirm this ratio remains to be seen.

SERIES 2. CONCENTRATED COMPOSTS

In placing any fertilizer upon a commercial basis, economy of production, a high grade of material and high degree of concentration are some of the essentials. It probably has been noted that one of the great disadvantages of the sulfur-floats method is the large amount of soil that has been used. Accordingly, it was thought advisable to try the effect of gradually decreasing the amount of soil until pure mixtures of sulfur and floats were reached. Four different kinds of mixtures were made in duplicate of about 100 gm. each. One of them contained 25 per cent of soil, another 10 per cent, another 2.5 per cent, while the fourth received no soil whatever. One set of the mixtures so prepared was used as a check, all moisture being withheld from them. It was also thought advisable to conduct another series, introducing some materials which might act as nutrients for the microörganisms, thereby intensifying sulfofication. Hence four more identical mixtures received additional treatments of 100 mgm. of ammonium sulfate, 50 mgm. of magnesium sulfate, and 25 mgm. of potassium chloride. With the exception of the checks the composts were inoculated with an infusion of a soil known to be high in sulfofying power, and the moisture maintained at an optimum content of 30 per cent throughout the incubation period. The composition of the composts and the amount of citrate-soluble P_2O_5 found in them at the end of 15 and 24 weeks are shown in table 3. Very gratifying indeed are the returns of this experiment, indicating as they do an intrinsic value for the method. The results obtained at the end of 15 weeks, although smaller, confirm those obtained at the end of the 24-week period. Hence a discussion of the results obtained for the longer incubation period only will be taken up.

As the sulfur and floats were in a very finely divided form, the composts were poorly aerated. As the results in series 5 show that aeration is a very important factor, a higher efficiency would probably have been obtained with the concentrated mixtures if they had been stirred at intervals. Considering

first compost 5, where we have the largest amount of soil (25 per cent), we observe an increase of 3073.33 mgm. of available P_2O_5 over the check. In no. 6, where the amount of soil is but 10 per cent, there is a decrease to 2498.00 mgm. of P_2O_5. A diminution of the soil in this instance seems to have been detrimental. In composts 7 and 8 there was still a decrease in available P_2O_5, the amounts being 2409.33 mgm. and 2369.57 mgm., respectively.

TABLE 3

Available phosphoric acid in the concentrated composts at the end of 15 and 24 weeks

LABORATORY NUMBER	TREATMENT	AMMONIUM-CITRATE-SOLUBLE P_2O_5		
		Average	Increase over check	Increase per gram of floats
	At end of 15 weeks			
		mgm.	*mgm.*	*mgm.*
1	25.0 gm. sulfur, 50 gm. floats, 25.0 gm. soil	535.00		
2	30.0 gm. sulfur, 60 gm. floats, 10.0 gm. soil	628.00		
3	32.5 gm. sulfur, 65 gm. floats, 2.5 gm. soil	673.83		
4	33.5 gm. sulfur, 67 gm. floats	690.10		
5	25.0 gm. sulfur, 50 gm. floats, 25.0 gm. soil	1703.33	1168.33	23.37
6	30.0 gm. sulfur, 60 gm. floats, 10.0 gm. soil	1690.00	1062.00	17.70
7	32.5 gm. sulfur, 65 gm. floats, 2.5 gm. soil	1809.17	1135.34	17.46
8	33.5 gm. sulfur, 67 gm. floats	1909.50	1219.40	18.20
9	25.0 gm. sulfur, 50 gm. floats, 25.0 gm. soil	2081.67	1546.67	30.93
10	30.0 gm. sulfur, 60 gm. floats, 10.0 gm. soil	1966.00	1338.00	22.30
11	32.5 gm. sulfur, 65 gm. floats, 2.5 gm. soil	2112.25	1438.42	22.13
12	33.5 gm. sulfur, 67 gm. floats	1916.20	1226.10	18.30
	At end of 24 weeks			
1	Composts 1 to 4 as above			
5	25.0 gm. sulfur, 50 gm. floats, 25.0 gm. soil	3608.33	3073.33	61.47
6	30.0 gm. sulfur, 60 gm. floats, 10.0 gm. soil	3126.00	2498.00	41.53
7	32.5 gm. sulfur, 65 gm. floats, 2.5 gm. soil	3083.16	2409.33	37.07
8	33.5 gm. sulfur, 67 gm. floats	3059.67	2369.57	35.37
9	25.0 gm. sulfur, 50 gm. floats, 25.0 gm. soil	3316.66	2781.66	55.63
10	30.0 gm. sulfur, 60 gm. floats, 10.0 gm. soil	3136.00	2508.00	41.80
11	32.5 gm. sulfur, 65 gm. floats, 2.5 gm. soil	3109.17	2435.34	37.47
12	33.5 gm. sulfur, 67 gm. floats	3044.03	2353.93	35.13

Composts 9 to 12 each received an addition of 100 mgm. of ammonium sulfate, 50 mgm. of magnesium sulfate, and 25 mgm. of potassium chloride.

Concerning ourselves now with the efficiency of the different mixtures as measured by the amount of P_2O_5 made available per gram of floats added, we notice that the highest efficiency was exhibited in no. 5, which received an addition of 25 per cent soil, in which instance there was secured 61.47 mgm. P_2O_5. No. 6 shows but 41.63 mgm. and no. 7 shows 37.07 mgm. of P_2O_5 for each gram of floats. The difference between these is not great, implying that a 2.5 per cent is about as good as a 10 per cent soil dilution. But either of

these does not far surpass the straight sulfur-floats compost which gives an efficiency of 35.37 mgm. Hence it appears that the latter would be a more economical compost than either nos. 6 or 7. The results obtained with the sulfur-floats combination look very favorable.

Taking up now the additional feature of the experiment, involving the addition of the nutrients for the microörganisms (nos. 9 to 12), it must be concluded that these materials did not exert any effect one way or the other, as the results secured are practically the same as those secured in treatments 5 to 8. This, however, is only one associative method tried out in an endeavor to secure maximum production of citrate-soluble P_2O_5 in concentrated composts; perhaps others to follow will be still more encouraging.

In a review of the experiment as a whole the striking feature is the persistency with which the composts maintain their available P_2O_5 output as the soil content diminishes. Even in the total absence of soil the results are particularly encouraging. In regard to the question as to whether sufficient P_2O_5 is made available to warrant the employment of concentrated composts, it must be admitted that the data at hand do not justify an affirmative answer. The present data do prove, nevertheless, that there is a possibility that some chemical treatment may be worked out, in which a stimulation of the bacterial activities will be brought about, which will in turn speed up the sulfofication processes.

SERIES 3. EFFECT OF SULFUR ON DIFFERENT INSOLUBLE PHOSPHATES

Since the common phosphate rocks vary considerably in their chemical composition and in their degree of hardness, obviously there would be a difference in the effect that sulfur would have upon floats derived from them. In making a study of this matter, a blue rock phosphate, a Florida soft rock phosphate and a Tennessee brown rock phosphate were employed. Also a sample of Vivianite, which was high in P_2O_5, and chemically pure tricalcic phosphate were used. Composts were made with 100-gm. portions of greenhouse soil. The amount of total P_2O_5 added in the various materials was ascertained, and a record kept of the same. The amounts of citrate-soluble P_2O_5 in the various composts at the end of 15 and 21 weeks are recorded in table 4. Without going into too much detail we shall merely make the observation that differences, as surmised, do exist between these materials.

In the case of the three natural tricalcic phosphates where approximately the same number of milligrams of phosphoric acid were contained in each compost, the Florida soft rock phosphate is evidently the most responsive to the action of sulfur oxidation. For the 15-week incubation period it gave an increase of 1757.50 mgm. of available P_2O_5 over the check as compared with 1278.75 mgm. for the blue rock phosphate and 1141.87 mgm. for the Tennessee brown rock phosphate. From the 15th to the 21st week the amount of available P_2O_5 in the composts made with the above phosphates increased to

3220.00 mgm., 2080.00 mgm. and 2073.12 mgm., respectively. These large increases might be partially explained by the fact that the composts were thoroughly stirred at the end of the 15th week in order to aerate them.

That the Florida soft rock phosphate is naturally more soluble than the others, is indicated by the fact that the untreated rock is more soluble in ammonium citrate solution than the other phosphate rocks. This somewhat natural inherent availability might partly explain the increased action. The larger amount of available P_2O_5 in the natural rock might also act as a stimulant to the sulfofying microörganisms. The softness of the Tennessee brown rock phosphate is probably of no avail, for it contains greater amounts of iron and aluminum which would tend to draw into an insoluble form a larger amount of the phosphoric acid rendered soluble through the agency of the

TABLE 4

Influence of sulfur oxidation on the availability of the phosphorus in different insoluble phosphates

PHOSPHATES EMPLOYED AND TREATMENT	AMOUNT OF P_2O_5 ADDED	CITRATE-SOLUBLE P_2O_5			
		End of 15 weeks		End of 21 weeks	
		Average	Increase over check	Average	Increase over check
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Blue rock phosphate	4410.00	93.75		87.50	
Blue rock phosphate, sulfur*	4410.00	1372.50	1278.75	2167.50	2080.00
Soft rock phosphate	4257 00	236.25		205.00	
Soft rock phosphate, sulfur	4257.00	1993.75	1757.50	3425.00	3220.00
Brown rock phosphate	4348.00	140.63		138.13	
Brown rock phosphate, sulfur	4348.00	1282.50	1141.87	2211.25	2073.12
Tricalcic phosphate, C. P	6864.98	2213.75		2398.75	
Tricalcic phosphate, sulfur	6864.98	4250.00	2036.25	4890.00	2491.25
Vivianite	636.00	566.25		631.25	
Vivianite, sulfur	636.00	782.50	216.25	745.00	113.75

* 5 gm. sulfur employed in each case.

sulfur. In the case of the blue rock phosphate its disadvantage is probably its inherent hardness, or rather its obstinacy to solubility. The action of sulfur is also manifested in the case of the C. P. tricalcium phosphate. Although its fine state of division gave rise to a large solubility without the use of sulfur, nevertheless it will be noticed that sulfur oxidation was very effective, increasing the amount of available P_2O_5 by 2036.25 mgm. in 15 weeks and to 2491.25 mgm. in 24 weeks. The effect upon Vivianite was not so marked because the check itself ran high, yet where sulfur was added, 745.00 mgm. of available P_2O_5 were secured at the end of 21 weeks. This apparent discrepancy is made clear when it is remembered, that the soil contains P_2O_5 which may be rendered soluble. This experiment, taken in its entirety, is of a suggestive value for the selection of raw phosphate rocks for use in com-

posts. It also shows the relation that sulfur bears toward the well-known commercial phosphates in the production of soluble P_2O_5. Other things being equal, it would seem that Florida soft rock phosphate is preferable under the conditions of this experiment to the others, for the maximum yield of P_2O_5 in the sulfur composts.

SERIES 4. THE EFFECT OF FINENESS OF DIVISION

In the matter of making phosphorus available by the use of sulfur, many somewhat attractive ideas suggest themselves, one of which is the thought of bringing the materials together in a fine state of division. Fineness from the standpoint of solubility is essential for some fertilizing substances—limestone and rock phosphate, for instance. Might not then, an intimate mixture of sulfur and floats likewise result in an increased action toward solubility? A trial of this was made in the following manner; 100-gm. portions of greenhouse

TABLE 5

Influence of fineness of division of the composts on the availability of phosphoric acid

COMPOST NUMBER	PLAN OF EXPERIMENT	CITRATE-SOLUBLE P_2O_5 IN COMPOSTS	
		Average	Increase over check
		mgm.	*mgm.*
1	15 gm. floats	138.13	
2	15 gm. floats, 5 gm. sulfur (added separately to soil and mixed)	2211.25	2073.12
3	15 gm. floats, 5 gm. sulfur (ground together and mixed with soil)	2430.00	2291.87
4	15 gm. floats, 5 gm. sulfur (ground with soil)	1380.00	1241.87
5	15 gm. floats, 2.5 gm. sulfur (added separately to soil and mixed)	1028.75	890.62

soil being used in each instance. In one case sulfur and floats were mixed with the soil by hand. In another the sulfur and floats were mixed together and were then passed through a mill before mixing with the soil. In still another the sulfur, floats and soil were ground up together in a mill, optimum moisture added and after incubating for 21 weeks, analyses were made. The results obtained are recorded in table 5.

It appears that an intimate mixture of the sulfur and floats before they are mixed with the soil, tends toward an increase of available P_2O_5. The difference is not very large but is appreciable, namely, 2291.87 mgm. as against 2073.12 mgm. where the sulfur and floats were not mixed before adding them to the soil. However, of most striking significance is the lessened yield of available P_2O_5 where the soil, floats and sulfur were ground together—striking because it helps to confirm results obtained in the following series, on aeration of the composts. In that series the tendency was toward a decrease of citrate-soluble P_2O_5 as the aeration was decreased. In the case at hand it will be readily grasped that the grinding of the soil would destroy its granular texture,

producing a powder which when moistened would lose what little air it might contain, thus creating an unfavorable environment for microörganisms. Hence we secure the low figure of 1241.87 mgm. of P_2O_5 rendered available. The fifth item in the table, in which 2.5 gm. of sulfur were employed, solicits no comment other than that this amount of sulfur is not sufficient to act on 15 gm. of floats. The main issue of the experiment, i.e., the test of fineness, would seem to be quite satisfactorily settled. We are forced to conclude that fineness or intimate contact will increase the availability of the phosphorus provided that the texture of the compost is not reduced to a fineness which will prohibit biological activities through lack of proper aeration.

SERIES 5. THE EFFECT OF AERATION AND INOCULATION

In experiments previously reported (47), the red silt loam soil, high in organic matter, was found to be more effective than the Sassafras soil, low in organic matter, as a medium for the production of available phosphorus from a sulfur-floats mixture. To assume definitely that this effectiveness was due to the presence of organic matter would be precipitate and possibly incorrect. Other factors might be involved, not the least of which would be the matter of inoculation. Soils might differ widely in regard to the presence of sulfofying microörganisms, the absence of which might prevent sulfofication regardless of the organic content of the soil. In this experiment a soil high in organic matter was employed, in order to eliminate it as the limiting factor. This condition was secured by incorporating 10 per cent of old composted manure with a red silt loam soil. One-hundred-gram portions were taken and composted with 5 gm. of sulfur and 15 gm. of floats, and moisture added in amount equal to 50 per cent of the water-holding capacity of the compost. These composts were made to differ in the matter of inoculation.

The inoculation was accomplished at the time the moisture was applied, 10 cc. of the water being added in the form of an infusion made up from a soil known to be efficacious in sulfofying action. It will be noted from the plan of the experiment that two further differences existed between the composts: namely, depth and aeration. At the end of the 15th and the 22nd weeks, determinations of the ammonium-citrate-soluble phosphoric acid were made and the results obtained are recorded in table 6.

It will be remembered that the organic matter content of these composts was the same, therefore the results should have corresponded, if organic matter is the controlling factor. On the contrary, very large differences were obtained. In every case at the end of 15 weeks the inoculated portions were higher in available phosphorus than the uninoculated, which is also true for the 22-week period. The highest amount of available P_2O_5 found in the inoculated portions at the end of 15 weeks was 1532.03 mgm., and the lowest in the inoculated portions was 465.15 mgm. Seven weeks later the ratio was 2960.75 mgm. to 503.25 mgm. The amounts cannot be given unqualifiedly,

however, for the depths of the composts were not the same, and hence aeration plays an important part. It will be noted, other things being equal, that the inoculated soil at the depth of $\frac{1}{4}$ inch was evidently the most favorable for the production of available P_2O_5, with the 2-inch depth a close second, the amounts of available P_2O_5 at the end of 22 weeks being 1460.75 mgm. and 1233.25 mgm., respectively. A depth of 4 inches appeared to be unfavorable. That this was due to aeration is indicated by the high results obtained where the com-

TABLE 6

Influence of aeration and inoculation on the accumulation of available phosphoric acid

TREATMENT	DEPTH OF COMPOST	AMMONIUM-CITRATE SOLUBLE P_2O_5	
		Average	Increase over check
At end of 15 weeks			
	inches	*mgm..*	*mgm.*
Check (initial P_2O_5)		156.10	
Inoculated	$\frac{1}{4}$	1145.00	988.90
Uninoculated	$\frac{1}{4}$	707.75	551.65
Inoculated	4	767.50	611.40
Uninoculated	4	621.25	465.15
Inoculated	2	996.50	840.40
Uninoculated	2	575.00	418.90
Inoculated and stirred*	2	1688.13	1532.03
Uninoculated and stirred	2	716.50	560.40
At end of 22 weeks			
Check (initial P_2O_5)		158.00	
Inoculated	$\frac{1}{4}$	1618.75	1460.75
Uninoculated	$\frac{1}{4}$	772.50	614.50
Inoculated	4	836.25	678.25
Uninoculated	4	661.25	503.25
Inoculated	2	1391.25	1233.25
Uninoculated	2	752.50	594.50
Inoculated and stirred	2	3118.75	2960.75
Uninoculated and stirred	2	2923.75	2765.75

* Stirred thoroughly every two weeks throughout period of experiment.

posts spread out in a 2-inch layer were stirred every two weeks. The high results obtained in the $\frac{1}{4}$-inch inoculated composts and the higher results secured in the frequently-stirred inoculated composts suggest that the oxidation of sulfur is brought about by aerobic microörganisms, and that the effect of inoculation is increased by aeration. This point would seem to be of practical importance, inasmuch as it indicates that composts made up in a commercial way would not be successful unless they were inoculated and also frequently turned. Aeration, however, without inoculation appears ineffective, as shown

by the fact that at the expiration of 15 weeks there were only 560.40 mgm. of available P_2O_5 in the uninoculated stirred composts, showing no increase over the other inoculated soils which were not stirred. But 7 weeks later 2765.75 mgm. P_2O_5 was found, which is a large increase and the second highest amount secured in the entire experiment. This is accounted for by the fact that the spatula used in stirring the composts was not sterilized, thus bringing about the inoculation of the composts intended to be uninoculated. Hence aeration alone was probably not the cause of this particular increase.

The results obtained in this experiment would make it appear that for a maximum production of available P_2O_5 in sulfur-floats-soil composts, inoculation and plenty of aeration are unquestionably advantageous and should receive foremost consideration.

SERIES 6. A STUDY OF METHODS OF INOCULATION

In the foregoing investigations the inoculation was accomplished by means of an infusion. For small composts this method has been quite satisfactory. For large composts, however, it might not be practicable. Hence the suggestion was presented of using soil from an old sulfur-floats compost heap as an inoculant. Years ago when gunpowder was produced through the medium of the so-called nitre composts, such a scheme was in vogue. On these nitre or saltpeter "plantations," whenever a new compost was started, it was the practice to incorporate with it a certain amount of inoculant secured from a compost that was known to have been productive. The success of this method at that time would indicate the success of such a method in the instance at hand. To give the matter a fair test, a soil was secured which had been composted with 5 per cent of sulfur for 15 weeks. And from this soil, composts made up with a red silt loam soil were inoculated with varying amounts of 1, 4, 7 and 10 per cent. The infusions also were made from the same soil, and each quantity of infusion added to the composts represented 5 gm. of soil. The amounts of citrate-soluble P_2O_5 obtained at the end of 15 and 24 weeks are recorded in table 7.

It will be noted in studying the results obtained at the end of the 15-week period that the increases of available P_2O_5 over the checks were much the same, the 7-gm. inoculations yielding the most, where both 2.5 and 5 gm. of sulfur were employed. The amounts 659.77 mgm. and 752.69 mgm. of P_2O_5, respectively, were not as great as that secured (849.33 mgm. P_2O_5) where the inoculation was made by infusion. Evidently, the infusion was more effective, possibly because it had a more distributive effect, i.e., penetrating to all parts of the compost. At the end of 24 weeks we find the composts inoculated with infusion still in the lead, with 1872.75 mgm. of available P_2O_5. But the composts inoculated with the soil were not far behind, as we obtained 1732.74 mgm. of P_2O_5 when 10 per cent of inoculant was employed, with 5 gm. of sulfur. Where 2.5 gm. of sulfur was used the total amounts of available P_2O_5 were

lower in every case than with 5 gm. of sulfur, the highest being 1501.05 mgm. with the 4 per cent inoculant. The inoculated composts were higher in every case than the uninoculated composts. Thus we see that the use of soil from old composts bids fair as a competitor for inoculation by infusion. While,

TABLE 7

Influence of different methods of inoculation on the availability of the phosphoric acid in the composts

LABORATORY NO.	TREATMENT	AT END OF 15 WEEKS		AT END OF 24 WEEKS	
		Average citrate-soluble P_2O_5	Increase over check P_2O_5	Average citrate-soluble P_2O_5	Increase over check P_2O_5
		mgm.	*mgm.*	*mgm.*	*mgm.*
1	1 per cent composted soil, 2.5 gm. sulfur	82.43		82.39	
2	14 per cent composted soil, 2.5 gm. sulfur	82.86		78.68	
3	7 per cent composted soil, 2.5 gm. sulfur	82.71		86.11	
4	0 per cent composted soil. 2.5 gm. sulfur	81.07		81.77	
5	1 per cent composted soil. 2.5 gm. sulfur, 15 gm. floats	669.06	529.67	1536.36	1395.73
6	4 per cent composted soil. 2.5 gm. sulfur, 15 gm. floats	701.28	561.89	1641.68	1501.05
7	7 per cent composted soil, 2.5 gm. sulfur, 15 gm. floats	799.16	659.77	1583.25	1442.62
8	10 per cent composted soil, 2.5 gm. sulfur, 15 gm. floats	703.76	564.37	1395.11	1254.48
9	1 per cent composted soil, 5 gm. sulfur	83.66		86.11	
10	4 per cent composted soil, 5 gm. sulfur	84.26		81.78	
11	7 per cent composted soil, 5 gm. sulfur	83.37		83.02	
12	10 per cent composted soil. 5 gm. sulfur	82.81		83.63	
13	1 per cent composted soil, 5 gm. sulfur, 15 gm. floats	882.17	742.78	1901.37	1760.74
14	4 per cent composted soil, 5 gm. sulfur, 15 gm. floats	884.65	745.24	1856.03	1715.40
15	7 per cent composted soil, 5 gm. sulfur, 15 gm. floats	892.08	752.69	1838.68	1698.05
16	10 per cent composted soil, 5 gm. sulfur, 15 gm. floats	835.09	695.70	1873.37	1732.74
17	No composted soil, 5 gm. sulfur	83.66		81.78	
18	No composted soil, 5 gm. sulfur, 15 gm. floats	728.53	589.14	1569.82	1429.19
19	No composted soil, 5 gm. sulfur, inoculated with infusion	83.77		83.02	
20	No composted soil, 5 gm. sulfur, 15 gm. floats, inoculated with infusion	988.72	849.33	2013.38	1872.75
21	No composted soil, 15 gm. floats	139.39		140.63	

of course it was evident that such should have been the case, judging from precedents and general principles, yet in the launching of a new enterprise like this it is usually imperative to secure permanent records of many apparently obvious reactions.

SERIES 7. INFLUENCE OF SOLUBLE PHOSPHORIC ACID

In previous experiments (47) it was shown that the sulfur was oxidized more rapidly when floats were present than in the sand alone. It was thought that the microörganisms responsible for the oxidation of the sulfur require more or less phosphorus for their life activities and hence were stimulated in their ability to oxidize sulfur. The purpose of this experiment, therefore, is to study the effect of small additions of soluble P_2O_5 on sulfur oxidation, with consequent action on the insoluble rock phosphate. Consequently, composts were made with 100-gm. quantities of greenhouse soil, 5-gm. quantities of

TABLE 8

Influence of soluble phosphoric acid on the accumulation of available phosphoric acid

TREATMENT	AVAILABLE P_2O_5	
	Average	Increase over check
End of 6 weeks		
	mgm.	*mgm.*
Floats	200.00	
Floats, 0.5 gm. acid phosphate	232.79	
Floats, sulfur	1871.89	1671.89
Floats, sulfur, 0.5 gm. acid phosphate	1853.84	1621.05
End of 12 weeks		
Floats	198.40	
Floats, 0.5 gm. acid phosphate	242.81	
Floats, sulfur	2962.29	2763.89
Floats, sulfur, 0.5 gm. acid phosphate	3132.74	2889.93
End of 18 weeks		
Floats	204.10	
Floats, 0.5 gm. acid phosphate	248.75	
Floats, sulfur	3375.00	3170.90
Floats, sulfur, 0.5 gm. acid phosphate	3538.30	3289.50

sulfur flour and 15-gm. quantities of Florida soft rock phosphate which contained 28.38 per cent total P_2O_5. All the composts were inoculated with 20 cc. of a 10 per cent infusion of an old compost; 33 cc. of water was added at the beginning of the experiment, and additional quantities of 2 cc. were added at end of every 5 weeks. The composts were stirred every two weeks. The results obtained are recorded in table 8.

There is very little difference between the available P_2O_5 content of the composts receiving no addition of available P_2O_5 (except that originally present in the floats) and that of the composts receiving 0.5 gm. of acid phosphate at the end of either the 6th, 12th or 18th week. For instance, at the end of 18 weeks with no available P_2O_5 added at the beginning we obtain an increase over the

check of 3170.90 mgm. of available P_2O_5; and with an addition of 0.5 gm. of acid phosphate we obtain 3289.50 mgm. of available P_2O_5. This increase is within the limits of experimental error, and hence one would not be justified in concluding that the soluble P_2O_5 has exerted an influence one way or the other.

TABLE 9

The influence of various salts on the availability of phosphoric acid of floats using sand as a medium

TREATMENT	AMMONIUM-CITRATE-SOLUBLE P_2O_5	
	Average	Increase over check
At end of 15 weeks		
	mgm.	*mgm.*
Sulfur	17.23	
Floats	131.55	
Sulfur, floats	591.08	459.53
Sulfur, floats, 0.20 gm. $(NH_4)_2SO_4$	616.60	485.05
Sulfur, floats, 0.50 gm. $(NH_4)_2SO_4$	751.75	620.20
Sulfur, floats, 0.20 gm. $NaNO_3$	195.55	64.00
Sulfur, floats, 0.50 gm. $NaNO_3$	166.70	35.15
Sulfur, floats, 0.20 gm. $MgSO_4$	613.38	481.83
Sulfur, floats, 0.50 gm. $MgSO_4$	638.13	506.58
Sulfur, floats, 0.20 gm. $MnSO_4$	345.56	214.01
Sulfur, floats, 0.20 gm. $MgSO_4$, 0.20 gm. $(NH_4)_2SO_4$	637.70	506.15
Sulfur, floats, 0.50 gm. $MgSO_4$, 0.50 gm. $(NH_4)_2SO_4$	699.63	568.08
At end of 22 weeks		
Sulfur	17.10	
Floats	130.75	
Sulfur, floats	771.25	640.50
Sulfur, floats, 0.20 gm. $(NH_4)_2SO_4$	783.75	653.00
Sulfur, floats, 0.50 gm. $(NH_4)_2SO_4$	855.00	724.25
Sulfur, floats, 0.20 gm. $NaNO_3$	197.75	67.00
Sulfur, floats, 0.50 gm. $NaNO_3S$	172.50	41.75
Sulfur, floats, 0.20 gm. $MgSO_4$	875.00	744.25
Sulfur, floats, 0.50 gm. $MgSO_4$	830.00	699.25
Sulfur, floats, 0.20 gm. $MnSO_4$	550.00	419.25
Sulfur, floats, 0.20 gm. $MgSO_4$, 0.20 gm. $(NH_4)_2SO_4$	808.75	678.00
Sulfur, floats, 0.50 gm. $MgSO_4$, 0.50 gm. $(NH_4)_2SO_4$	823.50	692.75

SERIES 8. EXPERIMENTS WITH POSSIBLE CATALYTIC AGENTS

Before the establishment of any new method it is often wise to subject it to many apparently irrelevant tests. Hence, while the sulfur-floats-soil composts have been shown to produce results, yet there might be a possibility that certain substances might accelerate the action, so that the floats could be rendered available in a shorter period of time. To this end experiments

were conducted in which white sand and a red silt loam soil were used as media. In the case of the white sand, composts containing 100 gm. of sand, 4 gm. of sulfur, and 15 gm. of floats were made, and 18 cc. of water added per compost—10 cc. of which was in the form of infusion, made from a soil which had been composted with sulfur several weeks. The miscellaneous materials introduced, and the results obtained at the end of 15 and 22 weeks are recorded in table 9.

It will be noted that sulfur and floats alone yielded an increase of 459.53 mgm. of available P_2O_5 at the end of 15 weeks and 640.50 mgm. at the end of 22 weeks. The 0.5-gm. portions of ammonium sulfate were slightly better, yielding 620.20 mgm. and 724.25 mgm. of available P_2O_5, respectively. The largest increase of available P_2O_5—744.25 mgm.—was obtained with the 0.2 gm. of magnesium sulfate at the end of 22 weeks. Manganese sulfate seemed to retard the sulfofication processes. On the whole, the results were much

TABLE 10

The effect of chemical treatment on the sulfur oxidized in the sand

TREATMENT	SULFUR OXIDIZED			
	Sulfur	Sulfur	Average sulfur	Increase over check
	mgm.	*mgm.*	*mgm.*	*mgm.*
Check (soil, sulfur, floats), at beginning	66.80	69.20	68.00	
Nitrogenous salts added				
None	825.55	828.00	826.78	758.78
0.20 gm. $(NH_4)_2SO_4$	798.00	801.40	799.70	691.26*
0.50 gm. $(NH_4)_2SO_4$	922.48	940.50	931.49	762.49*
0.20 gm. $NaNO_3$	192.00	191.48	191.74	123.74
0.50 gm. $NaNO_3$	123.20	125.10	124.15	54.15

* The amount of sulfur contained in the ammonium sulfate subtracted.

the same, and of no special importance except in the case where sodium nitrate was employed. Here even at the end of 22 weeks there was an increase over the check of only 67 mgm. of available P_2O_5 with 0.2 gm. of sodium nitrate, and 41.75 mgm. with the 0.5-gm. additions of sodium nitrate. This is very striking and should elicit more than passing comment, as it has been observed in the field by Vermorel and Danthony (73) and Lint (41) that sulfur did not produce beneficial results when sodium nitrate was added in connection with it as a fertilizer. From the amounts of available P_2O_5 obtained in this series it appears that sodium nitrate had a prohibitive effect upon sulfofication and that this might explain the results obtained by the above investigators. Consequently, the amount of sulfur oxidized was determined in the composts receiving no special treatment, sodium nitrate, and ammonium sulfate. The results are recorded in table 10. The data show conclusively that the addition of sodium nitrate to the composts of sand, sulfur and floats had a retarding effect upon the sulfofication processes. Of the

composts analyzed those receiving the 0.5-gm. additions of ammonium sulfate show the largest amount of sulfur oxidized, namely, 762.49 mgm. On the other hand, in those receiving additions of 0.20 gm. and 0.50 gm. of sodium nitrate, the amounts of sulfur oxidized were only 123.74 mgm. and 54.15 mgm., respectively. Whether the nitrate has an injurious effect upon the sulfofying microörganisms, whether it brings about conditions in which other organisms are able to thrive and hence crowd the sulfofiers out, or whether the latter organisms are able to utilize the sodium nitrate to the exclusion of the sulfur, are problems for further investigations.

Where the red silt loam was used as a medium the experiment differed only in the following respects: (a) 22 cc. of water was added to the composts, with

TABLE 11

Influence of various materials on the availability of phosphoric acid of floats when soil is used as a medium

TREATMENT	CITRATE-SOLUBLE P_2O_5			
	At end of 15 weeks		At end of 22 weeks	
	Average	Increase over check	Average	Increase over check
	mgm.	*mgm.*	*mgm.*	*mgm.*
Sulfur	46.53		51.25	
Floats	145.40		147.75	
Sulfur, floats	1069.03	923.63	1580.00	1432.25
Sulfur, floats, 0.20 gm. $(NH_4)_2SO_4$	921.03	775.63	1405.25	1257.50
Sulfur, floats, 0.50 gm. $(NH_4)_2SO_4$	994.85	849.45	1167.75	1020.00
Sulfur, floats, 0.20 gm. Mn SO_4	1161.25	1015.85	1547.50	1399.75
Sulfur, floats, 0.50 gm. Mn SO_4	967.18	821.78	1545.00	1397.25
Sulfur, floats, 1.00 gm. peat	1128.62	983.22	1447.50	1299.75
Sulfur, floats, 1.00 gm. peat, 0.20 gm. $MnSO_4$	1095.99	950.59	1337.75	1189.00
Sulfur, floats, 5.00 gm. peat, 0.20 gm. $MnSO_4$	1158.62	1013.22	1422.50	1274.75
Sulfur, floats, 2.00 gm. horse manure	1181.00	1035.60	1230.25	1082.50
Sulfur, floats, 5.00 gm. horse manure	1334.81	1189.41	1375.00	1227.25
Sulfur, floats, 2.00 gm. composted manure	818.87	673.47	869.25	721.50
Sulfur, floats, 5.00 gm. composted manure	953.55	808.15	1180.00	1032.25
Sulfur, floats, uninoculated	761.53	616.13	945.00	797.25

1 cc. additional for each gram of organic matter added; (b) further miscellaneous substances were employed; and (c) two of the composts were not inoculated. The results tabulated in table 11 suggest that organic substances such as peat and manure are undesirable for the most efficient production of available P_2O_5 in the composts made up with this soil as a medium. Manganese sulfate made a good showing for the 22-week period, but was not quite equal to the straight sulfur-floats-soil mixture. It is perhaps needless to give this experiment much discussion, as it is largely a verification of the preceding one. Attention might be called, however, to the low results secured where no inoculation was given.

The low results obtained in one instance where composted manure was used are not easily explained.

While the amount of citrate-soluble P_2O_5 formed in the red silt loam (table 11) is higher than in the sand, the indications of the point in question do not essentially differ. In fact, one experiment is a confirmation of the other. With the evidence at hand, it would appear that a straight sulfur-floats-soil compost is more effective than one to which the above substances have been added; for the materials here employed have not shown any markedly helpful or catalytic action beyond the limit of experimental error. On the contrary, one of them, nitrate of soda, has shown a strong depressing effect. This discovery of the action of nitrate of soda on sulfur oxidation will probably prove of commercial importance both in composting and where sulfur is applied to soil in the field.

SERIES 9. FURTHER STUDIES WITH POSSIBLE CATALYTIC AGENTS

As the foreign materials introduced into the sulfur-floats composts in the preceding experiments did not exert a stimulating action, this experiment was designed to study the effect of additional materials such as charcoal, ferrous sulfate, aluminum sulfate, etc. In this experiment composts were made up with 100 gm. of Sassafras loam soil, 5 gm. of sulfur and 15 gm. of Tennessee raw rock phosphate. Thirty cubic centimeters of water were added to each compost, 10 cc. of which were soil infusion.

The inoculation in this experiment differed, however, from that used in the previous experiment. The infusion in this case was from a soil which had been inoculated 8 weeks previously with soil from one of the very efficient composts made up with the greenhouse soil. To this soil had also been added small quantities of sulfur from time to time in order to ascertain if an efficient inoculating material could not be secured. In order to supply adequate aeration the composts were stirred every two weeks. The various materials added and the results obtained at the end of 9 and 15 weeks are recorded in table 12.

Taking into consideration the results obtained at the end of the 9-week period, it will be noted that the composts made up with sulfur-floats and soil alone yielded 1382.20 mgm. of available P_2O_5. This shows very clearly the high efficiency of the inoculating material because in another experiment where the same soil and same materials were used we obtained an increase of only 639.18 mgm. of available P_2O_5 in 30 weeks, or, in other words, we obtained 743.02 mgm. more available P_2O_5 in this experiment with no other treatment than inoculation in a period of 9 weeks than was obtained previously (47) in 30 weeks in the uninoculated soil.

Comparing the results obtained where the various materials were introduced with the untreated composts, it will be noted that three of the treatments have brought about large increases in the available P_2O_5. The addition of 0.02-

and 0.05-gm. quantities of ferrous and aluminum sulfate in combination, and a combination of 0.05-gm. quantities of ferrous sulfate, aluminum sulfate. and sodium silicate, brought about considerable increases in available P_2O_5

TABLE 12

Influence of ferrous sulfate, aluminum sulfate and other material: on the accumulation of available phosphoric acid

SPECIAL TREATMENT	AMMONIUM-CITRATE-SOLUBLE PHOSPHORIC ACID					
	End of 9 weeks			End of 15 weeks		
	Average	*Increase over check	*Increase due to treatment	Average	*Increase over check	*Increase due to treatment
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
Check (floats†)	111.25			120.50		
Check (floats, 0.0 2gm. $FeSO_4.7H_2O$)	138.75			126.25		
Check (floats, 0.02 gm. $Al(SO_4)_3.18H_2O$)	126.25			117.50		
Check (floats, 0.02 gm. $FeSO_4.7H_2O$, 0.02 gm. $Al_2(SO_4)_3.18H_2O$)	117.50			124.75		
None (floats and sulfur only)	1505.63	1382.20		2945.00	2822.75	
0.20 gm. calcium sulfate	1470.00	1346.57		2935.00	2812.75	
0.50 gm. calcium sulfate	1373.75	1250.32		2585.00	2462.75	
0.20 gm. powdered charcoal	1688.75	1565.32	183.12	2490.00	2367.75	
0.50 gm. powdered charcoal	1742.50	1619.07	236.87	3396.25	3274.00	451.25
0.02 gm. $FeSO_4.7H_2O$, 0.02 gm. $Al_2(SO_4)_3.18H_2O$	2261.83	2138.40	756.20	4162.50	4040.25	1217.50
0.05 gm. $FeSO_4.7H_2O$, 0.05 gm. $Al_2(SO_4)_3.18H_2O$	2221.75	2098.32	716.12	3788.75	3666.50	843.75
0.02 gm. $FeSO_4.7H_2O$, 0.02 gm. $Al_2(SO_4)_3.18H_2O$, 0.02 gm. $Na_2Si_4O_9$	1541.25	1417.82	35.62	3860.00	3737.75	915.00
0.05 gm. $FeSO_4.7H_2O$, 0.05 gm. $Al_2(SO_4)_3.18H_2O$, 0.05 gm. $Na_2Si_4O_9$	2108.13	1984.70	602.50	3542.50	3420.25	597.50
0.05 gm. $Al_2(SO_4)_3.18H_2O$	1661.25	1537.82	155.62	3636.25	3514.00	691.25
0.05 gm. $FeSO_4.7H_2O$	1678.75	1555.32	173.12	3777.50	3655.25	832.50
0.05 gm. $Na_2Si_4O_9$	1683.13	1559.70	177.50	3217.50	3095.25	272.50
0.02 gm. $KMnO_4$	1417.50	1294.07		2965.00	2842.75	20.00
0.01 gm. KI	1336.25	1212.82		1975.00	1852.75	
0.05 gm. KI	169.38	45.95		187.50	65.25	
0.01 gm. $CuSO_4$	1280.00	1156.57		2276.25	2154.00	
0.05 gm. $CuSO_4$	1266.25	1142.82		2683.75	2561.50	
0.01 gm. $ZnSO_4$	1757.50	1634.07	251.87	3136.25	3014.00	191.25
0.05 gm. $ZnSO_4$	1150.00	1026.57		2395.00	2272.75	

* Average of the four checks subtracted in each case.

† Floats contained 28.92 per cent of P_2O_5.

NOTE:—Checks received no sulfur.

over that found in the untreated composts. the amounts being 756.20 mgm., 716.12 mgm. and 602.50 mgm., respectively. With 0.20- and 0.50-gm. quantities of charcoal, 0.02-gm. quantities of ferrous sulfate, aluminum sulfate and sodium silicate in combination, and 0.05-gm. quantities of the three last-named

salts alone, and with 0.01 gm. of zinc sulfate, there is, apparently, a slight stimulating effect. All the other treatments brought about decreases of varying degree, the greatest retardation being caused by the 0.05 per cent addition of potassium iodide. In this case sulfofication processes were apparently stopped altogether.

The results obtained at the end of the 15-week incubation period show practically the same relative differences as those for the 9-week period, although of course, the amounts of phosphoric acid rendered available are greater. The addition of a combination of 0.02 gm. each of ferrous and aluminum sulfates has brought about an increase of 1217.50 mgm. of citrate-soluble phosphoric acid over the untreated composts, which is the largest increase for any of the treatments. In order to check the results obtained with the ferrous and aluminum sulfates in this experiment, the following investigation was carried out.

TABLE 13

Influence of ferrous and aluminum sulfates on the accumulation of available phosphoric acid

LABORATORY NUMBER AND ADDITIONS	CITRATE-SOLUBLE P_2O_5	
	Average	Increase over check*
	mgm.	*mgm.*
1. Floats (Tennessee rock 28.92 per cent P_2O_5)	121.38	
2. Floats, 0.02 gm. ferrous sulfate	138.75	
3. Floats, 0.02 gm. aluminum sulfate	126.25	
4. Floats, 0.02 gm. aluminum sulfate, 0.02 gm. ferrous sulfate	122.63	
5. Floats, sulfur	2437.50	2310.25
6. Floats, sulfur, 0.02 gm. aluminum sulfate	2927.50	2800.25
7. Floats, sulfur, 0.02 gm. ferrous sulfate	3196.25	3069.00
8. Floats, sulfur, 0.02 gm. ferrous sulfate, 0.02 gm. aluminum sulfate	3586.25	3459.00
9. Floats, sulfur, 0.04 gm. ferrous sulfate, 0.04 gm. aluminum sulfate	3526.25	3399.00

* Obtained by subtracting average amount of available P_2O_5 in composts 1, 2, 3 and 4.

In this experiment 5-gm. portions of sulfur flour and 15-gm. portions of Tennessee raw rock phosphate were added to 100-gm. portions of Sassafras loam soil. The moisture was maintained at 33 per cent and as in the preceding experiment the composts were stirred every two weeks. The citrate-soluble P_2O_5 was determined in the various composts at the end of 15 weeks. The composition of the composts and the amount of available P_2O_5 contained in them at the end of 15 weeks are recorded in table 13.

The addition of ferrous and aluminum sulfates, both alone and in combination, have brought marked increases in the available P_2O_5 of the composts. Thus we find an increase of 2310.25 mgm. of P_2O_5 in compost 5, which had received no addition of ferrous or aluminum sulfate, while in composts 6 and 7, to which had been added 0.02 gm. of aluminum sulfate and 0.02 gm. of ferrous sulfate, respectively, the amount of available P_2O_5 was increased, compost

6 yielding 2800.25 mgm., and compost 7, 3069.00 mgm. The largest increases, however, were brought about by the addition of the salts in combination. In this case we obtain an increase of 3459.00 mgm. and 3399.00 mgm. of available P_2O_5 when the salts were added in combination in 0.02-gm. and 0.04-gm. quantities, respectively.

The facts noted in the preceding experiment are thus amply confirmed. That the increase in availability of the composted floats is brought about by a stimulating of the sulfofying microörganisms rather than by a chemical reaction is clearly indicated. We find in composts 1, 2, 3, and 4 the available P_2O_5 to be 121.38, 138.75, 126.25 and 122.63 mgm., respectively. There was very little difference between the amount of available P_2O_5 in compost 1, which was composed of only floats and soil, and that in composts 2, 3 and 4 which were composed of floats, soil and ferrous or aluminum sulfate, or the two sulfates in combination. This shows that the phosphorus of the floats was not rendered available by any chemical reaction between the ferrous or aluminum sulfates and the floats.

Considered as a whole, the results obtained in this experiment and in the preceding one show that in order to obtain the maximum production of available phosphoric acid in the compost heap, in the shortest period of time, 0.4 pound each of ferrous and aluminum sulfates should be added to each ton of compost.

SERIES 10. THE EFFECT OF CALCIUM CARBONATE ON THE AVAILABILITY OF PHOSPHORUS

As the process of sulfofication is as yet not entirely understood and as there are, no doubt, many attending factors which influence it, it was thought expedient to study the effect of calcium carbonate. The influence of calcium carbonate has often been suggested, and Brioux and Guerbet (11), and Brown and Kellogg (13) found it in their experience to be a stimulant to sulfofication. An experiment was accordingly planned for this purpose. Sassafras loam soil in 100-gm. quantities was mixed with sulfur and floats and with calcium carbonate, and each compost inoculated with fresh soil infusion. For 12 weeks, optimum moisture conditions were carefully maintained. Then acidity and citrate-soluble phosphoric acid determinations were made. It will be noted that the experiment naturally divides itself into three parts. In the first, comprising composts 1 to 6, we have 0.25 gm. of calcium carbonate and 5 gm. of floats, with varying amounts of sulfur. In the second including compost 7 to 12 we have 0.5 gm. of calcium carbonate and 10 gm. of floats with varying quantities of sulfur. In the third group, composed of composts 13 to 16 there are fixed amounts of sulfur and floats with varying amounts of calcium carbonate.

The acidity and available phosphoric acid which were found at the end of the 12-week period are recorded in table 14.

Taking this experiment as a whole it cannot be considered entirely satisfactory either from the standpoint of the presence or absence of calcium carbonate. Possibly the experiment was not fair in regard to the length of time. A longer incubation period might have contributed altogether different results. And, although the treatment in regard to inoculation and moisture content, as well as to temperature and light, were apparently the same, yet possibly differences might have existed. Reviewing the results as a whole, when we consider the total increases and decreases, it is interesting to note that when calcium carbonate was added, there were five instances of increases in acidity totaling 202.00 cc. of N/50 KOH as against four decreases totaling 170.00 cc.

TABLE 14

Influence of lime on the accumulation of available phosphoric acid

LABORATORY NUMBER	TREATMENT	ACIDITY, CC. N/50 KOH	INCREASE IN ACIDITY DUE TO $CaCO_3$, CC. N/50 KOH	CITRATE-SOLUBLE P_2O_5	INCREASE IN P_2O_5 DUE TO $CaCO_3$
				mgm.	*mgm.*
1	2 gm. sulfur, 5 gm. floats	578.00		265.15	
2	2 gm. sulfur, 5 gm. floats, 0.25 gm. $CaCO_3$	800.00	22.00	273.82	8.67
3	4 gm. sulfur, 5 gm. floats	534.00		262.67	
4	4 gm. sulfur, 5 gm. floats, 0.25 gm, $CaCO_3$	596.00	62.00	158.59	− 4.08
5	5 gm. sulfur, 5 gm. floats	556.00		260.19	
6	5 gm. sulfur, 5 gm. floats, 0.25 gm. $CaCO_3$	596.00	30.00	280.01	19.82
7	2 gm. sulfur, 10 gm. floats	472.00		351.88	
8	2 gm. sulfur, 10 gm. floats, 0.50 gm. $CaCO_3$	500.00	28.00	361.79	9.91
9	4 gm. sulfur, 10 gm. floats	486.00		292.40	
10	4 gm. sulfur, 10 gm. floats, 0.50 gm. $CaCO_3$	452.00	−34.00	351.88	59.48
11	5 gm. sulfur, 10 gm. floats	578.00		433.65	
12	5 gm. sulfur, 10 gm. floats, 0.50 gm. $CaCO_3$	508.00	−70.00	361.79	−61.86
13	5 gm. sulfur, 15 gm. floats	526.00		436.87	
14	5 gm. sulfur, 15 gm. floats, 0.25 gm. $CaCO_3$	522.00	− 4.00	364.27	−72.60
15	5 gm. sulfur, 15 gm. floats, 0.50 gm. $CaCO_3$	586.00	60.00	416.30	52.03
16	5 gm. sulfur, 15 gm. floats, 1.00 gm. $CaCO_3$	524.00	−62.00	394.00	−22.30

For the phosphoric acid made available there were five cases of increases aggregating 159.84 mgm. It would appear, therefore, that in this experiment the presence of calcium carbonate exerted neither a marked beneficial nor a detrimental influence.

SERIES 11. EFFECT OF CALCIUM CARBONATE

As the results obtained in the preceding experiment were somewhat inconclusive, this experiment was planned with the idea of securing a check on the foregoing results as well as to determine the effect of larger quantities of lime as a stimulant to sulfofication processes.

One hundred-gram quantities of greenhouse soil, 5-gm. portions of sulfur

flour and 15-gm. portions of Florida soft rock phosphate were employed. Duplicate composts were made for each treatment. All the composts were inoculated with an infusion of a soil known to be very high in sulfofying power. The composts were stirred every two weeks. The plan of the experiment and the results obtained at the end of 8 and 15 weeks, respectively, are recorded in table 15.

In the composts which did not receive additions of calcium carbonate we note that from the 4354 mgm. of insoluble phosphoric acid there has been rendered available 1866.25 and 3468.75 mgm. of phosphoric acid at the end of the 8- and 15-week periods, respectively. At the end of 8 weeks the composts

TABLE 15

Influence of lime on the accumulation of available phosphoric acid

TREATMENT AND ADDITIONS	AVAILABLE P_2O_5	
	Average	Increase over check
At end of 8 weeks		
	mgm.	*mgm.*
Floats	200.00	
Floats, sulfur	2066.25	1866.25
Floats, sulfur, 0.20 gm. $CaCO_3$	1950.00	1750.00
Floats, sulfur, 0.40 gm. $CaCO_3$	2053.75	1853.75
Floats, sulfur, 0.60 gm. $CaCO_3$	2076.25	1876.25
Floats, sulfur, 0.80 gm. $CaCO_3$	2138.75	1938.75
At end of 15 weeks		
Floats	197.50	
Floats, sulfur	3666.25	3468.75
Floats, sulfur, 0.20 gm. $CaCO_3$	3305.00	3107.50
Floats, sulfur, 0.40 gm. $CaCO_3$	3442.50	3245.00
Floats, sulfur, 0.60 gm. $CaCO_3$	3551.25	3353.75
Floats, sulfur, 0.80 gm. $CaCO_3$	3355.00	3157.50

which had received 0.20-gm., 0.40-gm., 0.60-gm. and 0.80-gm. additions of calcium carbonate were found to contain practically the same amount of available phosphoric acid as the untreated, showing a slight increase where 0.80 gm. of calcium carbonate was added. At the end of 15 weeks, however, we find that the addition of calcium carbonate had brought about a decrease in available phosphoric acid in every case. The experiments thus show that lime is not a necessary adjunct to the sulfur-floats composting process.

SERIES 12. THE EFFECT OF PEAT

Among other things organic matter has in some instances suggested itself as an aid in accelerating the oxidation of sulfur in composts, thus making more of the phosphorus of floats available in a shorter period of time than in the

previous composting experiments. To give this a somewhat more thorough trial it was deemed advisable to try a partially mineralized form of organic matter in soil and sand. Since peat or its extractions are often used in biological experiments, and also in a commercial way as a medium for microorganisms, this material was selected. Composts were made up from Sassafras loam soil and white sea sand. The composts containing 4 gm. of sulfur and 15 gm. of floats were all inoculated with a soil infusion supposedly well supplied with sulfur oxidizers. Moisture conditions which were considered optimum were maintained for each treatment, 0.66 cc. of water being added extra for

TABLE 16

The available phosphoric acid in the composts as effected by peat

SOIL MEDIUM AND ADDITIONS	AT END OF 15 WEEKS	AT END OF 24 WEEKS
	Average Citrate-soluble P_2O_5	Average Citrate-soluble P_2O_5
	mgm.	*mgm.*
100 gm. soil, floats.*	152.25	138.55
95 gm. soil, 5 gm. peat	159.35	139.00
70 gm. soil, 30 gm. peat	247.85	187.80
100 gm. soil, floats, sulfur†	1616.50	3310.00
95 gm. soil, 5 gm. peat sulfur, floats	1534.29	2990.00
90 gm. soil, 10 gm. peat, sulfur, floats	1487.16	3147.50
80 gm. soil, 20 gm. peat, sulfur, floats	1128.08	2057.50
70 gm. soil, 30 gm. peat, sulfur, floats	1326.45	1801.00
No soil, 50 gm. peat, sulfur, floats	472.96	993.00
100 gm. sand, floats	146.10	125.05
95 gm. sand, 5 gm. peat	150.58	122.90
70 gm. sand, 30 gm. peat	215.36	166.88
100 gm. sand, floats, sulfur	567.74	828.50
95 gm. sand, 5 gm. peat, sulfur, floats	1083.02	1260.00
90 gm. sand, 10 gm. peat, sulfur, floats	1037.90	1327.50
80 gm. sand, 20 gm. peat, sulfur, floats	1096.40	1400.00
70 gm. sand, 30 gm. peat, sulfur, floats	1069.32	1576.25

* 15 gm. floats added in each case.

† 4 gm. sulfur added in each case.

each gram of peat. The arrangement of the series and the amounts of citrate-soluble P_2O_5 contained in them at the end of 15 and 24 weeks, respectively, are shown in table 16.

The analyses of ammonium-citrate-soluble P_2O_5 at the end of the 15- and 24-week periods show peat to have a widely different effect on the sulfofication processes in the loam soil and the sand. In the loam soil the highest results were obtained where peat was omitted at the end of both the 15- and the 24-week periods, the amounts of citrate-soluble P_2O_5 being 1616.50 mgm. and 3310.00 mgm., respectively. Where peat was used in the soil-sulfur-floats composts, there was a tendency toward a decrease of citrate-soluble P_2O_5

as the amounts of peat increased, until we get the lowest results of all where peat only was used; in this case only 993.00 mgm. of P_2O_5 from the floats was made available in 24 weeks.

In the case of the sand, just the opposite effects are noted. The lowest results occurred where no peat was added to the sulfur-floats combinations and while the production of available P_2O_5 at the end of the 15-week interval did not vary as the peat ratio varies, the tendency 9 weeks later was toward an increase of citrate-soluble P_2O_5 as the proportions of peat increased, 5 gm. of peat resulting in 1260.00 mgm., and 30 gm. of peat, 1576.25 mgm. of P_2O_5.

An important point that should be mentioned in this connection is that the increase brought about in the sea sand by the addition of peat is not an economical one, for the greatest yield of available P_2O_5 in the sand (1576.25 mgm.) is less than one-half the highest yield in the loam soil (3310.00 mgm.). Treatment for treatment, much more P_2O_5 is made available in the loam soil than in the sea sand. It appears that in the soil there are some favorable factors which do not exist in the sand, such as the ability to absorb certain toxic substances. Therefore peat, even where it did increase the quantity of available P_2O_5, was not in sufficient quantity to warrant its use. Thus this experiment strongly indicates that peat, from the commercial standpoint, at least, is not only an unnecessary but an undesirable adjunct to a sulfur-floats soil compost for the purpose of making available phosphoric acid.

SERIES 13. EFFECT OF PEPTONE ON THE AVAILABILITY OF PHOSPHORUS

In order to determine whether peptone would increase sulfur oxidation, and in this way increase the production of available phosphoric acid in the composts, 100-gm. quantities of Sassafras loam and greenhouse soil were each mixed with sulfur and floats. Both the Florida soft rock and the Tennessee brown rock phosphate were used. The ammonium-citrate-soluble phosphoric acid was determined at the end of 8 and 16 weeks, respectively. The composition of the composts and the average amount of available phosphoric acid contained in them at the end of these periods are given in table 17.

It will be seen from the results given in table 17 that a striking depression of the available phosphoric acid in the composts was caused by the presence of peptone. The addition of peptone has probably encouraged the development of certain species of bacteria which are not sulfofiers, and thus suppressed the active sulfofiers. Other reasons which could be advanced for this depression are: (a) the sulfofying microörganisms may not be able to develop where large quantities of soluble organic matter are present, being in this respect like the nitrifying bacteria; or (b) the species of microörganisms which are encouraged to develop may change the sulfur into a form other than sulfuric acid.

In order to determine what changes the sulfur in the compost had undergone, a qualitative test was made on all the composts. This test showed that

the sulfur was transformed only to sulfates in the composts in which peptone was absent. On the other hand, in the composts which had received 1 per cent of peptone, only a small amount of sulfates were present, although a

TABLE 17

Influence of peptone on the availability of floats in sulfur-floats-soil composts

COMPOSITION OF COMPOSTS	CITRATE-SOLUBLE P_2O_5 IN ENTIRE COMPOST			
	Sassafras loam		Greenhouse soil	
	Average	Increase over check	Average	Increase over check
	mgm.	*mgm.*	*mgm.*	*mgm.*
At end of 8 weeks				
15 gm. Florida soft rock phosphate	192.50		197.17	
15 gm. Florida soft rock phosphate, 1 gm. peptone	199.00		201.10	
15 gm. Tennessee brown rock phosphate,	125.75		130.00	
15 gm. Tennessee brown rock phosphate, 1 gm. peptone	135.10		136.10	
15 gm. Florida soft rock phosphate, 5 gm. sulfur	1515.57	1323.07	1983.54	1786.37
15 gm. Tennessee brown rock phosphate, 5 gm. sulfur	1490.54	1364.79	1610.00	1480.00
15 gm. Florida soft rock phosphate, 5 gm. sulfur 1 gm. peptone	220.92	21.90	206.67	5.57
15 gm. Tennessee brown rock phosphate, 5 gm. sulfate, 1 gm. peptone	149.65	14.55	125.90	−10.20
At end of 16 weeks				
15 gm. Florida soft rock phosphate	188.00		186.75	
15 gm. Florida soft rock phosphate, 1 gm. peptone	200.50		198.00	
15 gm. Tennessee brown rock phosphate	124.50		118.50	
15 gm. Tennessee brown rock phosphate, 1 gm. peptone	129.25		127.75	
15 gm. Florida soft rock phosphate, 5 gm. sulfur	3690.00	3502.00	3566.00	3379.25
15 gm. Tennessee brown rock phosphate, 5 gm. sulfur	3284.00	3159.50	3180.50	3062.00
15 gm. Florida soft rock phosphate, 5 gm. sulfur, 1 gm. peptone	218.50	18.00	178.50	−19.50
15 gm. Tennessee brown rock phosphate, 5 gm. sulfur, 1 gm. Peptone	155.25	26.00	200.00	72.25

large quantity of the sulfur had been changed into sulfites. Therefore, as no sulfites were present in the composts which had received no addition of peptone, it appears that the peptone has brought about conditions under which organisms which are able only to transform the sulfur into sulfites can develop.

SUMMARY

Under the conditions of the experiment the following conclusions appear to be justified.

1. A compost composed of 100 parts of soil, 120 parts of sulfur, and 400 parts of floats appears to be the most economical combination for the production of available phosphoric acid.

2. An appreciable amount of phosphoric acid is rendered available in composts consisting of only sulfur and floats, although the quantity is not so large as when soil is present. This indicates that a stimulation of sulfofication processes may be brought about by some treatment, and in this way enable us to obtain maximum results in composts without the aid of soil.

3. Of the natural tricalcium phosphates (Tennessee brown rock, Tennessee blue rock and Florida soft rock) Florida soft rock phosphate appears to be the most preferable for maximum yields of available phosphoric acid in composts containing sulfur.

4. Fineness or intimate contact of the constituents in composts will increase their efficiency, provided that the texture is not reduced to a fineness which will prohibit biological activities through lack of proper aeration.

5. The biological factor is influential in the oxidation of sulfur and the rendering available of phosphorus in floats. In inoculated composts, all other conditions being the same, the quantity of phosphorus rendered available in 9 weeks was double that made available in uninoculated composts in 30 weeks.

6. In making composts the question of aeration should receive foremost consideration. The results obtained in this series of investigations would make it appear that the microörganisms which oxidize sulfur are largely aerobic, and hence require an abundant supply of oxygen.

7. The presence of soluble phosphoric acid other than that contained in floats, will exert no influence on the production of available phosphorus in soil-sulfur-floats composts.

8. Ammonium sulfate, magnesium sulfate, calcium sulfate and zinc sulfate were found to exert no influence on the production of available phosphorus, while sodium nitrate, potassium iodide and copper sulfate exerted a marked depressing action. The addition of 0.2 per cent of sodium nitrate was found to inhibit sulfofication processes almost entirely.

9. Under certain conditions ferrous sulfate, aluminum sulfate, and a combination of the two salts exert a marked stimulating action on sulfur oxidation processes, when present in small amounts in a compost composed of soil, sulfur flour, and Tennessee brown rock phosphate. A mixture of the two sulfates caused the greatest stimulation. From the data which are advanced it appears that in composting under farm conditions, 0.4 pound each of aluminum and ferrous sulfates should be added to each ton of compost, in order to obtain a maximum production of available phosphorus.

10. Calcium carbonate may bring about a decrease in available phosphorus when added to a sulfur-floats-soil compost.

11. A compost is more efficient in producing available phosphorus in the absence of large amounts of organic materials, such as peat, fresh horse manure, old composted manure, and peptone. The more soluble materials will bring about the greatest decrease in efficiency.

12. Sulfofying microörganisms may develop more rapidly in the absence of soluble organic matter. They appear in this respect to be similar to the nitrifying organisms.

13. When peptone was added to a mixture of soil, sulfur and floats, sulfur was largely transformed into sulfites.

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TWENTY YEARS' WORK ON THE AVAILABILITY OF NITROGEN IN NITRATE OF SODA, AMMONIUM SULFATE, DRIED BLOOD AND FARM MANURES

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During the last twenty-five years the fertilizer industry in the United States has developed rapidly. From a comparatively small tonnage in the early nineties it has grown to more than 7 million tons in 1917.

As the industry has grown, the number of materials that go to make up the fertilizers has also increased greatly. Many by-products that were formerly allowed to go to waste are now carefully saved and worked up in the fertilizer factory. This is especially true of the nitrogenous materials, which, under normal conditions, form the most expensive part of the fertilizer.

The movement to save these waste materials containing nitrogen came none too early, for it was the depletion in the soil of this element that was largely responsible for the run-down and abandoned farms in the older sections of the United States. For this element, most crops show a quicker response than for any other, and conversely, a falling off in yield will come sooner with a deficiency of nitrogen than of any other element. A supply of available nitrogen aids the plant in getting a good start so that its leaves may begin early to elaborate food from the air and its roots may reach out for the water of the soil which holds in solution mineral plant-food.

Since nitrogen is supplied in many different forms, it at once becomes a question as to which of these is most efficient in crop production. Far too little attention has been given to this important question. Too often a certain material has been chosen because there was among farmers a general impression that this particular material was better than some other, when, as a matter of fact, there was no scientific basis for such conclusion. As an example, nitrogen from organic sources has been preferred by many because it was believed that organic matter thus supplied would be of great value in improving the physical condition of the soil, but in making this choice farmers overlooked the possibility of using a more readily-available material which would increase the crop residue sufficiently to more than make up for the small amount of organic matter contained in the few hundred pounds of dried blood, fish or tankage. Also, there is a widespread impression that the loss of nitrogen is greater when nitrates are used, than when organic nitrogen is used. But experiments both in this country and abroad show beyond a doubt that the

crop yields and the percentage of nitrogen recovered in the crop were greater (and hence the loss must have been less) when nitrates were used than when organic sources of nitrogen were used.

The question of availability of nitrogenous fertilizers began to receive serious consideration at several of the leading European experiment stations some 30 years ago and much valuable information has been accumulated by these stations.

About 10 years later the subject began to receive attention in this country and it is a satisfaction to find that the results obtained here are fairly in accord with the findings of the European investigators.

Fairly complete reviews of this early work have been given in recent publications (2, 3) and no attempt will be made here to cover this field.

The completion in 1917 of 20 years' work in which a comparison is made of the materials mentioned in the title of this paper would seem to justify the publication at this time of a brief summary of the work. A detailed account covering the first 15 years of this work has already been published (3). Much of this need not be repeated, but the results of the last 5 years are of value as confirming the earlier work.

EXPERIMENTAL

The work was originally outlined under the broad heading "Investigations Relative to the Use of Nitrogenous Materials," and this included: (a) a determination of the yield of dry matter and nitrogen in crops from soils receiving various treatments under controlled conditions; (b) the percentage of nitrogen in the crop as affected by the treatment; (c) the utilization of nitrogen in different materials; (d) the relative efficiency of nitrogen in different materials; (e) the residual effects of nitrogenous substances; (f) denitrification; and (g) the effect of special treatment on the income and outgo of nitrogen in the soil.

As the work has progressed, more and more attention has been given to the utilization and relative efficiency of nitrogen in different materials, and it is this phase of the work which is to receive consideration in this paper.

In order that the work might be under more perfect control, it was carried out in galvanized iron cylinders, open at both ends and having a diameter of 23½ inches and a depth of 4 feet. These cylinders were set in the ground so that about 2 inches remained above the ground level. Thus the contents of the cylinders are isolated so that the roots of the crops growing in them are prevented from getting mineral plant-food from outside sources. The subsoil is a gravelly sandy material such as occurs where the cylinders are located, but the top soil is a loam (Penn loam) brought from another source, an 8-inch layer of which was placed in each cylinder on top of the subsoil, each cylinder receiving the same weight of the thoroughly mixed soil.

When the work was begun all the soils were given a liberal treatment of lime in the form of ground limestone and with the exception of one series which

does not enter into this discussion, all have received annual dressings of acid phosphate and potassium chloride at the rate of 640 pounds and 320 pounds per acre, respectively. Thus nitrogen is made the limiting factor insofar as human control can provide. Various combinations of manure and fertilizer were arranged, but it is sufficient to report here only on the four nitrogenous materials mentioned in the title.

One series received the phosphoric acid and potash, but no nitrogen, in order that it might be used as a check. Thus if a certain amount of nitrogen is recovered in the crop from the nitrogen-treated cylinder, and it is desired to calculate the percentage of the applied nitrogen that was recovered, it is necessary first to deduct from the total amount of nitrogen recovered in the crop, the amount recovered from the check cylinder, and thus account for the soil nitrogen that the crop used.

It is at once obvious that this cannot be an absolutely correct method of determining the percentage recovered, since in those cylinders to which nitrogenous fertilizers have been applied, the plant will make a quicker start and the roots go farther in search of the nitrogenous materials of the soil than in the check cylinders where there is a pronounced deficiency of available nitrogen, and thus the check fails to be a *true* check. In this way it happens that the recovery may *apparently* be more than 100 per cent as shown in Series 8 B, for the years 1901 and 1910. However, there appears to be no way of overcoming this error so long as the work is carried out in the natural soil and if one starts with an artificial soil, other and more serious difficulties arise.

In this work no effort has been made to analyze the roots, since it would be well-nigh impossible to do this correctly, and even if it could be done the same error would be introduced. The roots and stubble are left just as under field conditions so that the residual effects of these may be observed.

To draw conclusions from 1 or 2 years of such work would be manifestly unfair, but when it is carried on for a period of 10 or 20 years, seasonal differences, differences due to the unequal decomposition of organic matter and differences due to slight errors, which are sure to creep in now and then, are largely smoothed out and results are obtained which can be accepted with a fair degree of confidence. The confidence in such results is strengthened when it is found that they check with similar work conducted in other places or even in other countries.

The work was started in these cylinders in the spring of 1898 with corn as the first crop in the rotation. Four 5-year rotations have been carried out as follows:

First Rotation	*Second Rotation*
1898.....Corn	1903.....Corn
1899.....Oats (millet)	1904.....Oats (corn)
1900.....Oats (corn)	1905.....Oats (corn)
1901.....Wheat	1906.....Wheat
1902.....Timothy (two cuttings)	1907.....Timothy (two cuttings)

Third Rotation		*Fourth Rotation*	
1908	Corn	1913	Corn
1909	Oats (corn)	1914	Oats (corn)
1910	Oats (corn)	1915	Oats (corn)
1911	Rye and oats	1916	Wheat
1912	Timothy (two cuttings)	1917	Timothy (one cutting)

The corn following the oats is grown as a residual crop (without further addition of fertilizers) to utilize any nitrogen which the oat crop may have failed to get. All corn is planted thick and harvested as forage rather than as mature corn. Oats are harvested as oat-hay just before maturity, and wheat is harvested at maturity and saved as grain and straw.

Nitrogenous materials are applied for each main crop in the rotation as follows:

Cylinder 4B, farm manure, at the rate of 16 tons per acre.
Cylinder 8B, nitrate of soda, at the rate of 320 pounds per acre.
Cylinder 17B, ammonium sulfate, equivalent to 320 pounds of nitrate of soda per acre.
Cylinder 18B, dried blood, equivalent to 320 pounds of nitrate of soda per acre.

Thus a careful record is kept of the amount of nitrogen applied each year and of the yield of dry matter from each cylinder. From determinations of the amount of nitrogen in the dry matter the total amount of nitrogen removed by the crop each year is easily calculated.

YIELD OF DRY MATTER

The yield of dry matter under the four different treatments for the 20 years is shown in table 1, averages being given for two 10-year periods and also for the entire 20 years. For each 10-year period the yield has been largest with the manure, though it is less for the second 10-year period than for the first, which would indicate that with manure at the rate of 16 tons per acre the fertility of the soil is not being fully maintained. The lowest yield is from 18B where dried blood is used as the source of nitrogen. Here again the average yield is less for the second 10-year period than for the first. For plots 8B and 17iB, which receive nitrate of soda and ammonium sulfate, respectively, the average yields for the first 10 years are essentially the same for the two treatments, but for the second 10 years the average for the nitrate of soda treatment is considerably above that for the ammonium sulfate; furthermore, the average yield with ammonium sulfate is, like the yield with dried blood and farm manure, less for the second than for the first 10-year period. With the nitrate of soda, however, the figures are reversed, that is, the average yield for the second 10 years is somewhat above that for the first 10 years.

The question may well be raised as to why the average yields on 4B, 17B and 18B should be less for the second 10-year period than for the first, while the yield on 8B has been well maintained for the 20 years. Since phosphoric

acid and potash have been supplied each year in liberal amounts, and lime has been used at stated intervals, it would seem clear that the falling off in yield must be due to a deficiency of available nitrogen, notwithstanding the fact that cylinders 17B and 18B receive each year just as much nitrogen as 8B, while 4B receives more than two and one-half times as much as 8B.

Data presented heretofore, and which are confirmed by results hereafter to be presented, show that of the four materials, nitrate of soda is most effective in crop production, that is, the crop is able to utilize or win back a larger percentage of nitrogen in this form than in any of the other forms. With a given amount of nitrogen, therefore, the crop yield can be better maintained over a period of years by the use of nitrogen in the form of nitrate of soda than in the other forms, provided the soil is one that does not allow rapid leaching.

TABLE 1

Yield of dry matter with different nitrogenous materials

FIRST 10-YEAR PERIOD						SECOND 10-YEAR PERIOD					
Year	Check*	4B	8B	17B	18B	Year	Check*	4B	8B	17B	18B
	gm.	gm.	gm.	gm.	gm.		gm.	gm.	gm.	gm.	gm.
1898	291.1	467.1	393.9	401.0	341.8	1908	169.0	326.0	331.0	286.0	228.0
1899	146.6	354.1	184.5	190.5	186.3	1909	164.0	208.0	244.0	217.0	218.0
1900	238.1	387.2	317.0	310.1	307.9	1910	214.0	422.0	338.0	287.0	276.0
1901	126.0	342.2	331.0	300.0	239.4	1911	68.0	236.0	160.0	117.0	126.0
1902	86.2	147.8	150.9	143.9	115.6	1912	88.0	221.0	187.0	153.0	115.0
1903	160.3	315.0	183.0	291.0	216.0	1913	177.2	390.5	312.5	228.5	286.5
1904	118.7	262.0	170.0	167.0	160.0	1914	137.0	285.8	222.4	196.9	198.3
1905	125.7	262.0	226.0	209.0	191.0	1915	103.7	231.2	211.0	178.3	147.5
1906	98.3	316.0	244.0	226.0	144.0	1916	91.4	250.9	217.3	181.6	112.9
1907	107.3	237.0	168.0	133.0	172.0	1917	71.1	229.0	208.0	167.0	139.0
Average†	149.8	309.04	236.83	237.15	207.4	Average‡	128.3	280.04	243.12	201.23	184.72

* Phosphoric acid, potash and lime; no nitrogen.

† First 10 years.

‡ Second 10 years.

This apparently is what has happened in this case. With the gradual exhaustion of *soil* nitrogen, which was made available by the use of lime, and the failure of the ammonium sulfate, blood and manure to give back in the form of crops as large a proportion of the applied nitrogen as the nitrate of soda, the yields with the former became gradually less.

The fact that cylinder 4B gave the largest average yield through 20 years must not be taken as meaning that the treatment given this cylinder is necessarily the best or most effective. It will be remembered that this cylinder receives cow manure at the rate of 16 tons per acre annually, the cost of which would be much in excess of the cost of 320 pounds of nitrate of soda or its equivalent in ammonium sulfate or dried blood, and therefore the larger

yield does not necessarily mean an efficient use of the applied nitrogen. As a matter of fact, the work shows this to be the least efficient of the four forms.

PERCENTAGE OF NITROGEN RECOVERED IN THE CROPS

Reference has already been made to the method of calculating the percentage of nitrogen that is recovered in the crop. The recoveries for the four different treatments covering the 20 years are shown in table 2. The averages for the period are as follows:

4B	32.69 per cent (manure)
8B	62.42 per cent (nitrate of soda)
17B	47.48 per cent (ammonium sulfate)
18B	38.69 per cent (dried blood)

This means that of 100 pounds of nitrogen applied in the four different forms, approximately one-third, three-fifths, one-half, and two-fifths, respectively, are recovered or won back in the crop.[1] As has already been mentioned these figures agree quite closely with results reported from European countries, and they also confirm earlier work carried out at this Station.

But even so, they are not satisfying figures. We at once ask why there is this enormous loss of nitrogen and especially why the loss is so much greater with the organic materials than with the nitrate of soda and ammonium sulfate. If the loss is to be attributed to the leaching out of the materials, then it would seem that the figures should be reversed. Unquestionably, a certain amount of loss takes place in this way, but this cannot explain the loss of over two-thirds from the manure against a little more than one-third from nitrate.

It is well known that organic materials must undergo certain transformations in the soil before the nitrogen can become available, and it seems that during these transformations nitrogen as ammonia, nitrate or as elemental nitrogen must be lost in considerable quantities. As bearing on this it may be pointed out that Russell and Richards (5) have shown by laboratory experiments with manure that in addition to the loss of ammonia by volatilization there is still a loss amounting to 15 per cent or more of total nitrogen, and they have gone further and shown that during decomposition there is an evolution of gaseous nitrogen. This they believe completes the account of the loss. This loss, they claim, does not go on under wholly anaerobic or wholly aerobic conditions but under mixed anaerobic and aerobic conditions which arise when manure is being produced. They explain further that in the natural manure heap nitrogen is also lost as gaseous ammonia as well as in the form of nitrogen gas.

[1] Or if we assign to nitrate nitrogen a value of 100, then the relative availability of the four materials stands as follows:

Nitrate of soda	100.0
Ammonium sulfate	76.1
Dried blood	62.0
Manure	52.4

It is very probable that in a more limited way, similar changes take place when organic compounds are placed in the soil and that a part of the loss of nitrogen noted in our experiments must be thus accounted for. It is a well-known fact that when an organic substance like cottonseed meal or dried blood is mixed with soil and incubated in the laboratory for a few days, escaping ammonia may be detected, and from this it is a natural conclusion that when large quantities of organic matter are placed in the soil under natural conditions, some ammonia will be lost by volatilization, especially when the temperature and moisture conditions are favorable. This then, together with the evolution of gaseous nitrogen, would in part at least explain the heavy loss of nitrogen where manure was used at the rate of 16 tons per acre.

TABLE 2

Percentage of nitrogen recovered from different materials

FIRST 10-YEAR PERIOD					SECOND 10-YEAR PERIOD				
Year	4B	8B	17B	18B	Year	4B	8B	17B	18B
1898	28.15	63.75	66.06	58.18	1908	16.97	42.77	24.20	27.38
1899	51.48	48.45	58.27	44.58	1909	18.25	80.64	54.94	49.04
1900	36.18	77.55	69.47	57.25	1910	54.74	110.74	62.12	51.22
1901	41.78	110.26	91.91	68.71	1911	20.98	64.10	48.46	41.59
1902	11.48	32.06	23.64	14.32	1912	29.11	49.16	27.45	10.96
1903	20.20	30.84	34.38	20.97	1913	27.63	32.92	15.50	40.26
1904	38.91	46.19	39.26	33.68	1914	52.46	74.35	67.86	56.55
1905	30.10	68.77	56.05	34.01	1915	32.13	64.10	52.53	48.12
1906	44.94	81.81	30.80	24.78	1916	36.60	68.96	57.53	20.26
1907	33.85	45.10	27.47	42.48	1917	27.95	55.77	41.75	29.41
Average *	33.71	60.48	49.73	39.90	Average†	31.68	64.35	45.23	37.48
Average ‡	32.69	62.42	47.48	38.69					

* First 10 years.

† Second 10 years.

‡ Twenty years.

A discussion of this subject would not be complete without a brief reference to the effect of cultivation on nitrogen losses.

Shutt[2] for example has shown that when the prairie soils of Saskatchewan were left undisturbed the loss of nitrogen was slight, but as soon as cultivation was commenced losses set in.

Russell (4) refers further to losses of nitrogen as follows:

> One of the Broadbalk wheat plots receives annually 14 tons of farm-yard manure per acre containing 200 pounds of nitrogen. Only a little drainage can be detected and there is no reason to suppose that any considerable leaching out of nitrates occurs, but the loss of nitrogen is enormous amounting to nearly 70 per cent of the added quantity.
>
> The condition for this decomposition appears to be copious aeration, such as is produced by cultivation and the presence of large quantities of easily decomposable organic matter.

[2] Cited by Russell (4).

Now these are precisely the conditions of intensive farming in old countries and of pioneer farming in new lands, and the result is that the reserves of soil and manurial nitrogen are everywhere being depleted at an appalling rate.

Russell refers to the recuperative actions that are going on, but says; "One of the most pressing problems at the present time is to learn how to suppress this gaseous decomposition and to direct the processes wholly into the nitrate channels."

In a paper on the nitrate content of cultivated and uncultivated soils, Blair and McLean (1), have called attention to the loss of nitrogen from cultivated soils and also to the low recovery from nitrogen applied as organic materials. They point out that land under cultivation is gradually being depleted of its store of nitrogen even when nitrogenous fertilizers are applied each year and that the average recovery of nitrogen applied in the form of fish scrap for a period of nine years, was only 36.36 per cent.

With the same nitrogen treatment soils allowed to run wild just about maintained their nitrogen content, while the carbon content of these soils was slightly increased.

The recovery of nitrogen in the four different treatments for the 20 years is shown by the curves in figure 1. A study of these curves shows that the high points are generally reached in either the first or second year of oats, and in the wheat year, while the low points occur almost invariably in the corn and timothy years. It is not entirely clear whether this is a seasonal variation or a crop characteristic.

It is certain, however, that the utilization of the residual nitrogen by the corn crops which follow the oats, helps to explain the high recovery for the years when oats are grown.

CONCLUSIONS

In a 5-year rotation on Penn loam soil well supplied with phosphoric acid, potash and lime, crop yields were better maintained over a period of 20 years with nitrate of soda at the rate of 320 pounds per acre than with an equivalent amount of ammonium sulfate or dried blood. For several years the latter gave results about on a par with the nitrate, but an average of the second 10-year period shows a considerable falling off with these materials as compared with the nitrate. This is no doubt due in part to the fact that the nitrate, being immediately available, gives the plant an early start which tends to keep it in the lead, and to the further fact that in the transformation of the ammonium salt and the organic material into nitrates, there is a considerable loss of nitrogen, possibly as ammonia gas or gaseous nitrogen or both. The loss cannot all be attributed to a leaching out of the materials, even though the nitrification of ammonia and organic residues may go on throughout a large portion of the year.

In the above-mentioned rotation cow manure at the rate of 16 tons per acre gave somewhat larger yields than nitrate of soda, but the increased yields were not sufficient to justify the increase in the cost of nitrogen.

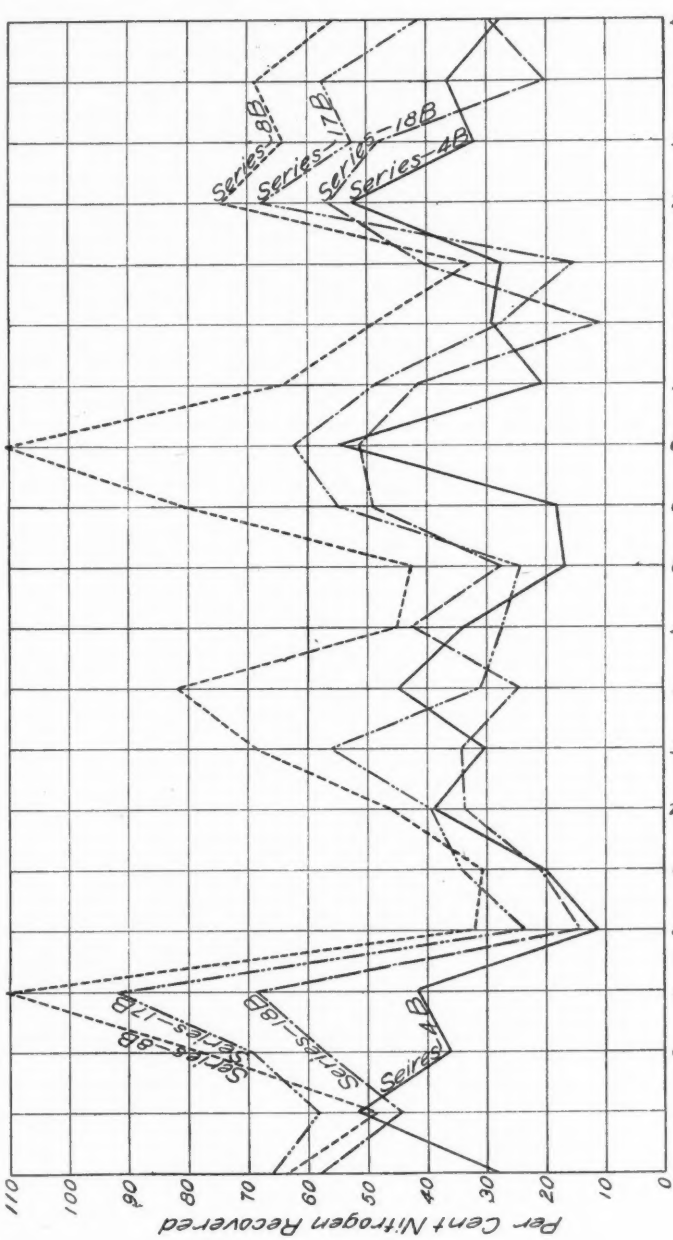

FIG. 1. DIAGRAM SHOWING RECOVERY OF NITROGEN FOR THE FOUR DIFFERENT TREATMENTS OVER A PERIOD OF 20 YEARS

Furthermore, the average yield with the manure was less for the second 10-year period than for the first, while the reverse is true with the nitrate of soda. Thus it is shown that with 16 tons of manure per acre annually, the crop yield is not being maintained, while with nitrate of soda at the rate of 320 pounds per acre annually it is increasing slightly, as shown by the average for the second 10-year period.

The percentage of nitrogen recovered in the crop was greater with the nitrate than with any of the other materials, the 20-year average being as follows:

	per cent
Nitrate of soda	62.42
Ammonium sulfate	47.48
Dried blood	38.69
Cow manure	32.69

The average recovery with nitrate for the second 10-year period was 64.35 per cent as against 60.48 per cent for the first 10-year period, whereas the average recovery with the ammonium sulfate, dried blood and manure were all less for the second 10-year period than for the first.

This is in agreement with the crop yields, and indicates a diminishing efficiency for the ammonium sulfate, blood and manure, and a gradual increase in efficiency for the nitrate of soda.

The work shows that when properly used nitrate of soda alone as a source of nitrogen may be depended upon to maintain crop yields over a long period, and that a given amount of nitrogen in this form is more effective than an equivalent amount in the form of ammonium sulfate, or organic materials.

Its effect is to produce larger crops per unit of nitrogen, and these crops, in turn, leave behind in the soil larger crop residues, and with carbonate of lime to aid in their decomposition these furnish a sufficient supply of organic matter to keep the soil in good physical condition.

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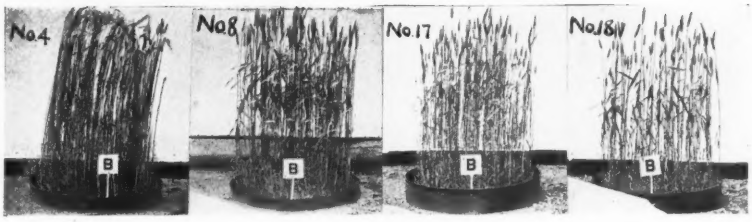

FIG. 1. WHEAT 1916

No. 4, cow manure, 16 tons per acre; No. 8, nitrate of soda, 320 pounds per acre; No. 17, ammonium sulfate equivalent to the nitrate; dried blood equivalent to the nitrate.

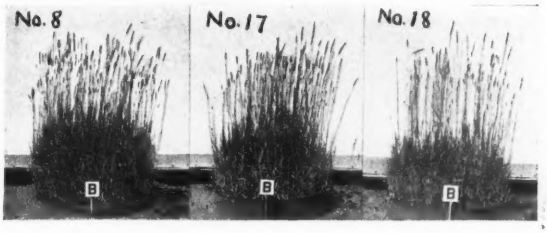

FIG. 2. TIMOTHY 1917

No. 8, nitrate of soda as above; No. 17, ammonium sulfate as above; No. 18, dried blood as above. (No photograph secured of No. 4 in 1917.)

A METHOD FOR THE COUNTING OF CERTAIN PROTOZOA IN THE SOIL

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With the recent developments in soil protozoology, various methods for counting protozoa have been devised as well as means of cultivation. The chief methods in use at the present time are five in number, namely: (a) the direct counting of a drop; (b) the dilution method of Rahn (6); (c) the agar-plating method of Killer (2); (d) the use of the standard loop of Müller (5); (e) the use of the blood counting apparatus as described by Kopeloff, Lint and Coleman (4). This last is perhaps the most recent. A review of the above methods is given by Kopeloff and Coleman (3).

In connection with our work on soil protozoa the last-named method seemed very satisfactory, although difficulties were encountered under certain conditions. The authors found the accuracy of the method to be impaired (a) when the number of organisms in the sample fell below four hundred per cubic centimeter of suspension, and (b) when by any chance soil particles, the diameter of which was greater than 0.1 mm., had been carried into the chamber. The probability of error due to settling while taking the sample will be discussed and demonstrated in the data.

To meet our conditions the following method was devised and was based on the principles of the Sedgwick-Rafter cell,[1] commonly used in the estimation of plankton in water examination, in conjunction with vital stain and a semi-solid diluent.

APPARATUS

The cell was of the type described by Whipple (7). The cell used was made by E. A. Thompson, of Amherst, and consisted of a large slide 75 by 25 mm. upon which was cemented a brass plate exactly 1 mm. in thickness, having in the center a rectangular opening 50 by 20 mm. A cover slip of the same size was used, thus giving a cell of 1 cc. capacity.

MICROSCOPES

In the course of the work two microscopes were used, a Leitz and a Bausch and Lomb binocular. The Leitz was used with the no. 3 objective and no. 4 ocular, this combination having the following physical constants:

[1] The use of this cell was suggested by Mr. Arthur W. Walker of the Northwestern University Medical School.

Numerical aperature	0.30
Initial magnification	10.3 mm.
Working distance	5.5 mm.
Diameter of visible field	2.1 mm.
Equivalent focus	16.2 mm.
Magnification	103×

The Bausch and Lomb binocular was used in the greater part of the work. In all cases the oculars were 10× and the objectives either 24-mm. or 40-mm. The former gave a magnification of 74× and the latter a magnification of 34×. With the first-named the diameter of the visible field was 2 mm., with the latter it was 4.25 mm.

CHEMICALS

The vital stain was the indicator phenolsulphonephthalein. It was procured in ampoules as sold for injection for the renal efficiency test and used without altering its condition.

The diluent consisted of a 4 per cent solution of gelatine (Gold Label), the reaction of which had been adjusted to P_H 8 approximately by the addition of N/5 NaOH.

METHOD OF PROCEDURE

For test work the organisms were cultivated in mannite solution (1). Samples of soil were put in the medium, usually about 5 gm. of soil to 100 cc. of medium and then the mixture was gently shaken. This was allowed to stand for two to three weeks at room temperature and usually at the end of this time was found to contain an abundance of organisms.

The counting, unless otherwise designated, was carried on in the following manner:

The cell was thoroughly cleaned with water and wiped with xylene. By means of a platinum loop the bottom was covered with a thin layer of the phenolsulphonephthalein solution. This was allowed to dry and the culture, previously diluted with the alkaline gelatine solution, was run in after the manner described by Whipple (7). The mixture was then counted and from these data in the usual manner the total number of organisms per cubic centimeter of suspension was determined,

In the work to determine the relation of time of settling to the total number of organisms, the same procedure was followed throughout with the exception that 1 gm. of sand was added before the dilution was made up to 10 cc. and the whole gently shaken until the sand was evenly distributed. The time of taking the samples is given in the data.

DATA

I. Count made by Sedgwick-Rafter method without vital stain or sand

A culture of large ciliates was taken and the counts were made with the Bausch and Lomb binocular with 10× oculars and 40-mm. objectives. The number of fields to total area of cell was 81.9.

COUNT	FIELDS										AVERAGE	TOTAL	D*	D^2†
	1	2	3	4	5	6	7	8	9	10				
1	1	4	5	2	2	1	1	5	3	4	2.8	229.0	36.0	1,296.0
2	2	3	0	2	2	2	2	5	3	2	2.3	188.0	5.0	25.0
3	1	2	0	1	4	1	1	7	1	4	2.2	180.0	13.0	169.0
4	1	6	1	4	4	3	0	2	0	1	2.2	180.0	13.0	169.0
5	1	3	2	4	2	1	3	4	3	2	2.3	188.0	5.0	25.0
Sum												965.0		1684.0
Mean												193.0		

* The deviation from the mean.

† Square of the deviation from the mean.

$$E_S = \pm 0.6745 \sqrt{\frac{1684.0}{5}}$$

$$= \pm 12.37$$

Percentage of error = $12.37 \div 193.0 \times 100 = 6.41$

The probable error for a single counting is calculated according to the usual formula:

$$E_g = \pm 0.6745 \sqrt{\frac{\Sigma D^2}{n}}$$

where E_s is the error of single variate,
0.6745 is a constant for standard deviation.
ΣD is the sum of the squares of the deviations from the mean,
and "n" is the number of variates.

It will be noted that in the above results there is considerable error. This seems to be due to the fact that the protozoa were not defined and therefore some may not have been seen.

II. A count of small flagellates as in I

Unstained and without sand. A dilution of 1:20 was used. The microscope was binocular with 10× oculars and 24-mm. objectives. There were 318.5 fields, in the total area of the cell.

COUNT	FIELDS										AVERAGE	TOTAL/20	D	D^2
	1	2	3	4	5	6	7	8	9	10				
1	2	2	2	2	3	4	1	0	0	1	1.7	541.4	19.14	366.33
2	1	2	3	0	3	3	1	1	0	0	1.4	445.9	114.64	13,142.30
3	1	4	2	0	1	0	1	3	3	2	1.7	541.4	19.14	366.33
4	4	3	2	0	3	1	2	1	2	0	1.8	573.3	12.76	162.33
5	4	1	2	3	2	2	2	3	1	2	2.2	700.7	140.16	19,648.00
Sum												2,803.7		33,685.7
Mean												560.54		

$$E_S = \pm 0.6745 \sqrt{\frac{33685\ 78}{5}}$$

$$= \pm 55.35$$

Percentage of error = $55.35 \div 560.54 \times 100 = 9.87$

The results indicate that the probable error increases considerably in this case. It may be due to the size of flagellates, and also to higher magnification which means a small visible field.

III. A count of large ciliates with Sedgwick-Rafter method and the use of vital stain

Binocular used with 10× oculars and 40-mm. objectives; dilution 1:1.

COUNT	FIELDS										AVERAGE	TOTAL	D	D^2
	1	2	3	4	5	6	7	8	9	10				
1	3	2	1	1	1	2	1	4	0	0	1.5	246	4	16
2	2	1	2	1	2	1	1	3	0	2	1.5	246	4	16
3	2	2	2	1	0	2	1	0	2	2	1.4	230	12	144
4	1	2	1	0	3	0	1	2	3	2	1.5	246	4	16
Sum												968		192
Mean												242		

$$E_s = \pm 0.6745 \sqrt{\frac{192}{4}}$$

$$= \pm 4.6710$$

Percentage of error = $4.6710 \div 242 \times 100 = 1.93$

Calculating from these data it may be seen that the totals check very closely and the percentage of error is reduced considerably. In the experimental work it was found that the organisms were clearly defined.

IV. Count of small flagellates using vital stain but no sand

The binocular was used with 10× oculars and 24-mm. objectives. Suspension was diluted 1:20.

COUNT	FIELDS										AVERAGE	TOTAL/20	D	D^2
	1	2	3	4	5	6	7	8	9	10				
1	4	3	2	0	2	2	1	0	2	2	1.8	573.3	19.14	366.3
2	2	2	2	3	0	1	1	0	3	3	1.7	541.4	2.76	7.6
3	1	3	0	3	0	3	2	1	3	3	1.9	605.1	50.94	2,594.9
4	5	2	1	1	1	2	0	3	1	1	1.7	573.3	19.14	366.3
5	3	1	1	2	3	2	1	1	0	1	1.5	477.7	76.46	5,846.0
Sum												2,770.8		9,181.1
Mean												554.16		

$$E_s = \pm 0.6745 \sqrt{\frac{9181.1}{5}}$$

$$= \pm 28.79$$

Percentage of error = $28.79 \div 554.16 \times 100 = 5.21$

Calculating as in the case of III we find a lower percentage of error than in the unstained preparations, although it is higher than in III. There seems to be but little doubt that this is due to the use of the stain.

V. Indicating the influence of sand upon the counting during the time of settling

A culture of large ciliates was used in connection with the proposed method and samples of 1 cc. were taken at 5-minute intervals starting with the sand, 60–80 mesh, still in suspension. Binocular with 10× oculars and 40-mm. objectives were used.

COUNT	FIELDS										AVERAGE	TOTAL	D	D^2
	1	2	3	4	5	6	7	8	9	10				
1	2	1	1	1	2	3	2	1	3	1	1.7	139.0	83.4	6,955.60
2	0	1	2	1	0	1	0	1	0	3	0.9	74.0	18.4	337.79
3	0	0	0	2	1	0	0	0	1	0	0.4	33.0	22.6	510.76
4	0	0	0	1	0	0	1	0	0	0	0.2	16.0	39.6	1,568.20
5	1	0	0	1	0	0	0	0	0	0	0.2	16.0	39.6	1,568.20
Sum												278.0		10,940.55
Mean												55.6		

$$E_S = \pm 0.6745 \sqrt{\frac{10940.55}{5}}$$

$$= \pm 31.55$$

Percentage of error = $31.55 \div 55.6 \times 100 = 56.87$

Calculations show the effect of time of settling in relation to the total number of organisms per cubic centimeter and the error resulting from this cause is very large, as indicated above.

VI. Check count on V

Same organisms and technique employed as in the case of V. In place of one sample of 10 cc., in this case 5 tubes were made up as in V and all were shaken simultaneously and the samples taken as noted above. Thus an error from change of dilution was avoided.

COUNT	FIELDS										AVERAGE	TOTAL	D	D^2
	1	2	3	4	5	6	7	8	9	10				
1	2	1	1	2	1	1	3	2	1	1	1.5	123	74	5,476
2	2	1	0	0	0	1	1	1	1	1	0.8	65	16	256
3	0	1	1	0	0	0	1	0	1	0	0.4	33	16	256
4	0	0	0	1	0	0	0	0	0	1	0.2	16	33	1,089
5	0	0	0	0	0	0	0	0	0	1	0.1	8	41	1,681
Sum												245		8,758
Mean												49		

$$E_S = \pm 0.6745 \sqrt{\frac{8758}{5}}$$

$$= \pm 28.23$$

Percentage of error = $28.23 \div 49 \times 100 = 57.62$

Here the result of the preceding experiment is duplicated, indicating the very high probable error.

VII. Time of settling in relation to number of small flagellates

The tubes were used in the manner described under VI. One gram of sand was placed in each tube and the total volume made up to 10 cc. with a mixture consisting of a 1: 5 dilution of the culture. The objectives used on the binocular were 24-mm.

COUNT	FIELDS										AVERAGE	TOTAL/5	D	D^2
	1	2	3	4	5	6	7	8	9	10				
1	2	9	12	4	8	9	4	4	3	7	6.2	1,974.6	923.6	853,005
2	8	3	5	2	3	5	3	3	3	2	3.7	1,178.4	127.4	16,268
3	4	3	5	3	2	2	1	3	3	2	2.8	891.8	159.2	26,539
4	4	3	3	5	1	1	1	1	1	2	2.2	700.6	350.4	125,860
5	2	2	1	1	1	3	2	1	2	1	1.6	509.6	541.4	293,110
Sum												5,255.0		1,314,782
Mean												1,051.0		

$$E_s = \pm 0.6745 \sqrt{\frac{1314782}{5}}$$

$$= \pm 345.88$$

Percentage of error = $345.88 \div 1051.0 \times 100 = 32.09$

The above shows quite clearly that small flagellates are affected in much the same way as the ciliates.

VIII. Check count on VII

Same technique as VII, except that a 1: 10 dilution was used.

COUNT	FIELDS										AVERAGE	TOTAL/5	D	D^2
	1	2	3	4	5	6	7	8	9	10				
1	1	3	4	4	4	2	2	2	0	1	2.3	1,465	726.2	527,375
2	2	1	2	1	2	1	3	0	1	0	1.3	828	89.2	7,966
3	1	1	1	2	1	1	0	1	1	1	1.0	637	101.8	10,366
4	1	0	1	0	1	1	0	1	1	1	0.7	446	292.8	85,732
5	1	1	0	0	1	0	2	0	0	0	0.5	318	420.8	177,050
Sum												3,694		808,489
Mean												738.8		

$$E_s = \pm 0.6745 \sqrt{\frac{808489}{5}}$$

$$= \pm 27.23$$

Percentage of error = $27.23 \div 738.8 \times 100 = 36.69$

This count seems to check the results found in VII.

SUMMARY AND CONCLUSIONS

Percentage of probable error

LARGE CILIATES				SMALL FLAGELLATES			
Without sand		With sand		Without sand		With sand	
−Stain	+ Stain	- Stain	+ Stain	- Stain	+ Stain	- Stain	+ Stain
6.5	1.93*	56.87	57.62	9.87	5.21*	32.09	36.69

* Percentage of probable error for the proposed method obtained under the conditions described previously.

− signifies without stain.

+ signifies with stain.

It is the authors' experience that the above data seem to indicate several interesting points, namely:

1. The use of a vital stain aids materially in defining the organism.
2. The use of the semi-solid diluent inhibits the rapid motion,
3. The results of a count are seriously affected when the soil is allowed to settle.
4. The soil particles do not seem to affect the accuracy of the counts, especially when the vital stain is used.
5. The probable error seems to vary inversely with the size of the organisms and directly with the magnification.

The method is presented, not with the intention of supplanting methods already in use, but rather with the idea of supplementing certain methods which, within definite limits, give excellent results.

The method has its limitations as the authors well realize. The cell does not permit a magnification that will include all the protozoa in the soil, neither does it allow for the possibility of the organisms being concealed by the soil particles. Nevertheless, the data given above seem to show that under the stated conditions gratifying results were obtained.

The advantages of the method may be summed up in the following statements:

1. The sample counted consists of a comparatively large amount of soil dilution, therefore allowing the counting to be done while the soil is in suspension.
2. The vital stain brings the organisms in the field into prominence.
3. The use of the semi-solid diluent prevents rapid motion and lessens the chance of error.
4. The procedure is simple and quick.
5. The apparatus is inexpensive.

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SULFOFICATION IN RELATION TO NITROGEN TRANSFORMATIONS

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At present considerable interest is taken in the use of sulfur in agriculture. Experimental evidence has been obtained which indicates that finely-ground sulfur added to some soils has favorably influenced plant growth; and that the acidity produced by the process of sulfur oxidation has a remarkable solvent action on mineral plant nutrients, including phosphates, thereby converting them into more available forms.

In considering the possibilities of elementary sulfur as a means for either directly or indirectly increasing crop production, some questions arise as to the effect of the acidity produced on nitrogen transformations in acid and basic soils. Preliminary data pertaining to the relations between sulfofication and the production of ammonia and nitrates under controlled conditions are presented.

EXPERIMENTAL PROCEDURE

Description of soils

The soils used in the experimental work were representative of three distinct types and provided for wide variations in content of organic matter and basicity.

The silt loam soil which is deficient in calcium carbonate and organic residues has a total lime requirement of approximately 4000 parts of calcium carbonate per million. The black clay used is decidedly basic in reaction, although it contains only 300 parts of calcium carbonate per million.

The peat was thoroughly decomposed and required approximately 10,000 parts of calcium carbonate per million to satisfy its requirements for base.

Five hundred-gram portions of the soils reduced sufficiently to pass through a 2-mm. sieve were used as a basis for the different treatments.

Soil treatment

The additions where made were flowers of sulfur, casein, rock phosphate, and calcium carbonate. Sulfur and rock phosphate were supplied at the rate of 1000 parts per million; and the quantity of casein added furnished 500 parts of nitrogen per million.

For the purpose of studying the effect of a supply or deficiency of a readily salifiable base on the processes involved, the additions of calcium carbonate provided for different degrees of basicity. In the case of the acid silt loam the largest addition of calcium carbonate, 4000 parts per million, was slightly in excess of the amount necessary to satisfy completely the soil's requirement; one-half and one-fourth this amount also were added to certain mixtures of the silt loam soil.

The natural basicity of the black clay was increased by 1000 and 500 parts of calcium carbonate per million. The largest addition of calcium carbonate to the decidedly acid peat was 10,000 parts per million and the smallest 2000.

When the materials were mixed with the soils, water was added to satisfy 60 per cent of their water-holding capacity. The several mixtures were transferred to quart jars and placed in a darkened cabinet, the temperature of which was maintained at 28° to 30°C.

The total weights of the jars and contents were obtained at the beginning of the period and weighings made at regular intervals when the water lost by evaporation was replaced; weighings were made and water added every fourth day.

Optimum conditions for aeration prevailed, as the containers were uncovered and the mixtures stirred two days after each addition of water required to adjust their water content.

Fifty different mixtures were included in the experiment, duplicate jars of each being prepared on the same date. The interval between the beginning of the experiment and the time when the transformations which had occurred were determined was 17 weeks.

ANALYTICAL METHODS

At the end of the experimental period the amount of sulfur as sulfate, of nitric and ammoniacal nitrogen, the acidity and the alkalinity were determined in the water extract of the mixtures, according to the following methods.

Solution

At the end of the incubation period the contents of the jars were emptied into a large porcelain evaporating dish, thoroughly mixed, and returned to the jars as quickly as possible to prevent loss of weight by evaporation. A weighed portion of the mixture corresponding to 400 gm. of dry soil was transferred to wide-mouth bottles having a capacity of 4000 cc., and water added so that the total volume in contact with the soil was 2500 cc.

The extraction was continued through a period of 14 hours with shaking at regular intervals during the first two hours. Filtration was made through a battery of Berkfeld filters employing pressure secured from an automatically controlled pump. The first portion of the filtrate was discarded. Approximately 2000 cc. of solution were secured in each case.

Nitrogen as ammonia in water solution

A 200-cc. portion of the filtered solution was transferred into a Kjeldahl flask and a half gram of freshly calcined magnesium oxide added. The ammonia distilled off was passed through a second and smaller Kjeldahl flask used as a scrubber and from this into a 500-cc. Erlenmeyer flask made of Pyrex glass. All connecting tubing was made of Pyrex glass. The liberated ammonia was absorbed in 1/10 normal acid and titrated with 1/20 normal alkali, methyl red being used as an indicator.

Nitrate nitrogen

Nitrogen in this form was determined by the Devarda reduction method as modified by Allen (1).

The solution remaining after distilling off ammonia with magnesium oxide was diluted with 200 cc. of distilled water, free from ammonia and nitrates, 2 cc. of 50 per cent sodium hydroxide solution were added and the contents of the flask boiled for 20 minutes. The flasks were then cooled, 2 gm. of Devarda's alloy added, and at once connected to the same apparatus as was used for the ammonia determination, and boiled for 40 minutes.

Sulfates

Water-soluble sulfates were determined gravimetrically in 200-cc. aliquots of the filtered solution after first acidifying with hydrochloric acid and boiling to decompose carbonates before the addition of barium chloride.

Acidity and alkalinity

The acidity or alkalinity of the water extract of the mixtures expressed in terms of sulfuric acid or calcium carbonate was determined by titrating a 100-cc. aliquot of the solution as follows:

Ten cubic centimeters of N/50 H_2SO_4 were added, the solution boiled and cooled, and then titrated with N/50 NaOH solution, phenolphthalein being used as an indicator.

SULFUR OXIDATION

The results for soluble sulfur as sulfates extracted by water from the differently treated soils are included with the data for nitric and ammoniacal nitrogen, and acidity and basicity of the water solutions, in the accompanying tabulations.

The amounts of water-soluble sulfates obtained from those mixtures in which no sulfur was included were practically the same. This indicates that a greater proportion of the natural sulfur supply has been oxidized in the acid silt loam than in the basic clay, since the total sulfur content of the basic clay

TABLE 1

Sulfur and nitric and ammoniacal nitrogen in acid silt loam. Data expressed as parts per million of soil

TREATMENT	$CaCO_3$ ADDED	SULFUR	NITRIC NITROGEN	AMMONIACAL NITROGEN	ACIDITY AS H_2SO_4	ALKALINITY AS $CaCO_3$
None		20	43	0	0	9
Floats		18	77	0	0	25
Floats	4,000	52	116	0	0	212
None	4,000	58	103	0	0	140
Casein		44	200	132	61	0
Casein	4,000	56	440	0	0	56
Casein, floats		32	186	55	24	0
Casein, floats	4,000	59	400	11	0	87
Sulfur	4,000	671	88	0	0	12
Sulfur	2,000	675	22	33	52	0
Sulfur	1,000	710	11	46	223	0
Sulfur		718	8	33	511	0
Sulfur, floats	4,000	524	96	10	0	75
Sulfur, floats	2,000	553	29	38	30	0
Sulfur, floats	1,000	589	18	43	196	0
Sulfur, floats		775	12	48	666	0
Sulfur, casein	4,000	661	295	4	18	0
Sulfur, casein	2,000	808	92	241	76	0
Sulfur, casein	1,000	740	33	208	200	0
Sulfur, casein		725	10	328	144	0
Sulfur, floats, casein	4,000	646	296	88	Neutral	
Sulfur, floats, casein	2,000	752	98	254	79	0
Sulfur, floats, casein	1,000	683	44	208	128	0
Sulfur, floats, casein		716	44	288	159	0

TABLE 2

Sulfur and nitric and ammoniacal nitrogen in basic black clay. Data expressed as parts per million of soil

TREATMENT	$CaCO_3$ ADDED	SULFUR	NITRIC NITROGEN	AMMONIACAL NITROGEN	ALKALINITY AS $CaCO_3$
None		38	142	11	90
Floats		31	141	11	93
None	1,000	38	143	11	106
Floats	1,000	35	143	0	175
Sulfur		656	107	11	78
Sulfur, floats		790	144	10	78
Sulfur	1,000	811	145	11	43
Sulfur	500	707	146	10	93
Sulfur, floats	1,000	760	158	11	46
Sulfur, floats	500	723	145	16	90

TABLE 3

Sulfur and nitric and ammoniacal nitrogen in acid peat. Data expressed as parts per million of soil

TREATMENT	$CaCO_3$ ADDED	SULFUR	NITRIC NITROGEN	AMMONIACAL NITROGEN	ACIDITY AS H_2SO_4
None		118	255	259	353
Floats		108	210	252	412
None	10,000	108	556	122	137
Floats	10,000	87	560	115	39
Sulfur		937	105	357	294
Sulfur, floats		952	100	392	353
Sulfur	10,000	818	112	399	274
Sulfur	5,000	942	80	406	294
Sulfur	2,000	981	58	398	372
Sulfur, floats	10,000	1025	108	318	235
Sulfur, floats	5,000	935	98	420	118
Sulfur, floats	2,000	881	105	403	235

is about 500 parts per million and the silt loam contains 230. Apparently, conditions are more favorable for oxidation of the natural supply of the silt loam, or the sulfur in these soils, differing widely in their characteristics, is present in different forms. No doubt a considerable proportion of the sulfur in soils which, like the black clay, are distinguished by their high organic matter content, exists as organic combinations.

Peat soils generally contain considerable amounts of soluble sulfates of calcium and magnesium; consequently, the larger amounts of soluble sulfur obtained from the peat can not be regarded as an indication of conditions being much more favorable for the oxidation of its original sulfur content.

In all the mixtures where sulfur was included in the treatment, there has been a considerable production of sulfates, the amount of sulfur oxidized depending upon the soil and the treatment. Somewhat larger amounts of sulfates were extracted from the peat to which sulfur was added.

The quantities of sulfates found in the water extract of the acid silt loam are of interest as an index of the effect of the wide range of treatment, especially the different degrees of basicity provided, on the sulfur oxidation process. Although the amounts of sulfates resulting from oxidation of sulfur compounds naturally present in the acid silt loam are small as compared with the quantities found in the water extract of soils to which sulfur was added, they furnish an indication of the influence of differences in treatment on sulfofication.

Where sufficient calcium carbonate was present to satisfy the soil's requirement for base an increase of soluble sulfur was found. The addition of casein has also produced the same effect.

Considering in detail the results for the sulfur group of mixtures, including those with floats and casein in addition, it will be noted that different amounts of calcium carbonate have had an appreciable influence on the degree of sulfofication.

Where the treatment was sulfur, or sulfur and floats, the minimum oxidation of sulfur occurred when the total requirement of the soil for base was satisfied by calcium carbonate supplied at the rate of 4000 parts per million of soil. The sulfur as sulfates has tended gradually to increase with the lesser additions of calcium carbonate, being greatest where no basicity was supplied.

It will be noted that in the group of mixtures having casein with sulfur, the effect of the several amounts of calcium carbonate has been different. The indications furnished by the sulfur results when casein was included are that the optimum basicity for sulfofication was one-half the total lime requirement, the most soluble sulfur being found where 2000 pounds of calcium carbonate were added, rather than where no calcium carbonate was supplied, as was the case in the sulfur and the sulfur and floats groups of mixtures.

Rock phosphate, or floats, appears to have had a slight effect on sulfur oxidation when calcium carbonate was added. Comparing the sulfur results through the three applications of calcium carbonate in the mixtures receiving sulfur, and sulfur and floats, it will be observed that when floats were included the oxidation of sulfur has been slightly depressed.

ACIDITY AND ALKALINITY

From 50 to 80 per cent of the sulfur added having been oxidized, the acidity produced will have had an effect on other processes.

The figures for acidity stated as sulfuric acid show that it follows the order of the sulfur oxidized, gradually increasing with the smaller additions of base, and as would be expected, is greatest where no calcium carbonate was supplied to neutralize the acidity.

This relation between the maximum oxidation of sulfur and acidity exists only where casein was not included in the mixtures with sulfur. With casein and sulfur both added to the soil, the acidity where no calcium carbonate was present was decidedly less than that of corresponding mixtures without casein, the acidities of the sulfur mixtures being 511 and 666, while those of the mixtures which included casein with sulfur were 144 and 159 parts per million. This depression of the acidity of the water extract in the one case is evidently due to the influence of sulfur oxidation in limiting the transformation of proteid nitrogen so that it proceeded to ammonia only. Acidity being produced by the oxidation of sulfur, and no calcium carbonate being supplied, the basicity of the soil was not sufficient for the further change from ammoniacal to nitric nitrogen. Consequently, the increased accumulation of ammonia where casein was present has neutralized the acidity which has been produced through sulfofication.

While considerably more nitrogen as ammonia was found in the sulfur-casein mixtures than where casein was not included with sulfur and additions

of 2000 and 1000 parts per million of calcium carbonate were made, the acidity of the water extract has not been changed appreciably.

The alkalinity of the water extract, of course, was greatest where calcium carbonate was furnished to satisfy the total requirement of the soil, and nitrification and sulfofication proceeded less actively than when either casein or sulfur was present. It will be noted that this alkalinity has been decidedly decreased where the largest formation of nitrates from proteid nitrogen occurred, the amounts of nitric nitrogen being 400 and 440 parts per million. The calcium in solution as calcium nitrate was also greatly increased where the alkalinity was decreased.

The figures for alkalinity and soluble calcium, considered in relation to the maximum production of nitrates, strikingly illustrate the base requirement of the nitrifying process. Further indications of the effect of nitrification in depleting the soil's supply of bases is furnished by the increased acidity of the water extract of the soil mixture in which nitrogen supplied by casein has been partially changed to both ammoniacal and nitric forms in the absence of sufficient calcium carbonate for the more complete transformation to nitrates.

The solutions from the black clay were all alkaline. While the amount of sulfur oxidized in the clay soil mixtures was approximately the same as in the acid silt loam, the natural basicity of the clay was in excess of the amount of base attacked by acidity resulting from sulfofication. The water extracts from the peat were all acid regardless of sulfur oxidation.

NITRIFICATION

The chief purpose of the investigation which furnished the reported data has been to determine the effect of sulfofication on nitrification in acid and basic soils, and whether these processes have selective power to combine with specific soil constituents. While no attempt has been made to study the phenomena of nitrification and ammonification, the results have contributed some interesting information pertaining to the influence of different treatments on these processes.

The nitrogen data show that the transformation of proteid nitrogen in different types of soils is influenced by several factors. Considering first the nitrate nitrogen in the soils to which no nitrogen was added, it will be observed that the largest accumulation was in the acid peat; a smaller amount was found in the basic clay and the minimum production occurred in the acid silt loam. These figures parallel the total nitrogen content of the three soils regardless of their extreme variations in basicity and acidity. The nitrification process does not appear to have been greatly depressed in the decidedly acid peat. In this connection, mention is made of the fact that samples of acid peats taken during the summer months when conditions were favorable for nitrification showed a nitrate content in field soils of 200 parts per million.

Nitrification as an indicator of acidity or basicity of soils is quite strikingly

illustrated by the production of nitrates in the naturally basic black clay, as compared with that in the silt loam, which has a total requirement of approximately 4000 pounds of calcium carbonate per million pounds of soil.

The amounts of nitric nitrogen in the black clay mixture were practically the same whatever the treatment, while in the acid silt loam nitrification was decidedly increased when calcium carbonate was added. No mixtures of the black clay or peat included casein as part of the treatment on account of their natural supply of organic nitrogen; consequently, the comparison with respect to relations between basicity and nitrification is made between the silt loam receiving no casein and the other soils.

The maximum production of nitrate nitrogen in the acid silt loam occurred where calcium carbonate was added in amount sufficient to satisfy the soil's requirement for base. This accumulation of nitrate nitrogen equal to more than 80 per cent of the organic nitrogen added can not be regarded as the total quantity of the added nitrogen which has been changed during the whole period, since ammonification when casein or blood is the source of nitrogen may proceed more rapidly than the ammonia produced can be converted to nitrates, with the probability of there being a considerable loss of nitrogen.

The effect of increasing basicity is quite pronounced in the peat soil where no oxidation of added sulfur occurred. While the addition of calcium carbonate sufficient to supply the lime requirement of the peat soil has caused the largest production of nitrate nitrogen, this increase is only about double that in the peat receiving no calcium carbonate or other treatment.

The nitrogen results when sulfur has been oxidized in the silt loam mixtures receiving the smallest addition of calcium carbonate, one thousand parts per million, which is one-fourth the amount required to satisfy the lime requirement, show that this amount of base has very little effect on nitrification, since the amount of nitric nitrogen was practically the same as that recovered from the soil with its requirement entirely unsatisfied. This is so where casein was present as well as where the indications were obtained from the natural nitrogen supply of the soil.

Acidity developed by sulfofication has had a decided effect on the nitrogen transformation, especially in the acid silt loam where the greater variety of treatment including proteid nitrogen supplied by casein affords an opportunity for making more extended comparisons.

The nitrogen results for this soil have clearly shown that a measure of either the nitric or ammoniacal nitrogen independently would not have furnished correct indications of changes from proteid to other forms of nitrogen when oxidation of added sulfur has occurred, for the reason that with certain conditions of treatment no appreciable amounts of nitrates were produced, the transition being to ammonia only, while under other conditions the change has proceeded more completely and practically all of the nitrogen was present as nitrates.

The transformation from ammonia to nitrates is depressed in the silt loam when sulfur has been oxidized, unless the acidity produced has been neutralized by calcium carbonate. This was also true for the nitrates in the acid peat. Sulfofication has had very little influence on nitrification in the basic clay, the quantities of nitrates found being practically the same regardless of sulfur oxidation.

One of the interesting points brought out by the work on nitrogen is the quantitative relationship between the results for nitrates and the calcium carbonate additions to the silt loam where the indications were obtained from the natural nitrogen supply as well as where proteid nitrogen was supplied by casein.

When sufficient calcium carbonate was present to satisfy the soil's requirement, excluding that necessary for the neutralization of acidity developed by sulfofication, the maximum quantities of nitrates were produced, the quantity found gradually decreasing with the smaller additions of carbonate and the ammoniacal nitrogen increasing. Although calcium carbonate in amount considered to be sufficient to satisfy the soil's demand for base exclusive of the additional requirement resulting from the oxidation of sulfur, increased the production of nitrate nitrogen where sulfur and casein were included together this quantity of calcium carbonate, however, was not adequate to satisfy the requirement of the soil for base and at the same time neutralize the acidity produced by the oxidation of sulfur. Evidence of this is furnished by the amounts of nitrates recovered from the mixtures which included casein with and without sulfur, and additions of calcium carbonate made at the rate of four thousand pounds per million. Where no sulfur was added, the quantity of nitrates recovered was 440 parts per million and where sulfur was oxidized there was a decrease to 295 parts per million.

AMMONIFICATION

When the results for nitrogen as ammonia are studied in relation to the nitric nitrogen figures obtained under the conditions of this experiment, they are of special significance in that they show the influence of various factors on the transition from the one form to the other.

Excluding the mixtures of peat soil, the quantities of ammonical nitrogen found are relatively small as compared with the ammonia extracted from the soil mixtures which included casein and sulfur. Oxidation of sulfur in the naturally basic clay soil has produced no variation in the amounts of ammonia, the slight differences exhibited by the results being well within the limit of analytical error. This uniformity conforms with the regularity observed between the results for nitrates and ammonia throughout the acid silt loam group of mixtures, that is, when conditions were made favorable for the production of nitrates by supplying base, the amounts of ammonia were small.

One relation between the two forms of nitrogen which is shown by the results is that the amounts of ammoniacal nitrogen vary inversely with the nitrates.

Casein being included in the silt loam mixtures and provision made for different degrees of basicity, the results for these mixtures are of interest when considered in detail.

It will be noted by referring to the data included in table 1, that the maximum accumulation of ammonia in the sulfur-casein treated mixtures receiving 4000, 2000 and 1000 pounds of calcium carbonate per million, is in direct relation to the largest amount of sulfur oxidized. Nitrogen as ammonia and sulfur as sulfate in these groups of mixtures were recovered in largest amount where calcium carbonate was added at the rate of two thousand parts per million of soil.

Where acidity was produced from sulfofication and no calcium carbonate was available to neutralize it, the transition from proteid to other forms of nitrogen proceeded to a slight extent only beyond ammonia, as the conditions were not favorable for the further change to nitrates and only small amounts of nitrogen in this form were found.

The results for ammoniacal nitrogen in these mixtures compared with the figures for acidity furnish evidence that in the absence of calcium carbonate, the ammonia produced neutralizing the sulfuric acid formed was combined as ammonium sulfate, since the acidity of the water extract was decidedly less than was found for similar mixtures where ammonifying organisms did not have a supply of proteid nitrogen to transform into ammonia. However, the acidity figures when basicity conditions were apparently optimum for sulfofication do not indicate that the ammonia formed in largest amounts from casein has materially changed the acidity from that where no casein was included in the mixtures with sulfur. The increased quantity of soluble sulfates associated with the ammonia present where two thousand parts per million of calcium carbonate were provided may be partly ammonium sulfate.

In these cases where conditions resulting from sulfofication, and the presence of insufficient base were such that the transformation from ammonia to nitric nitrogen was largely prevented, there would undoubtedly be a fixation of a part of the ammonium sulfate formed, since it is an established fact that soils have the capacity for fixing considerable amounts of ammonia from added ammonium salts. It would seem that following the fixation of ammonia from ammonium sulfate formed, whether this phenomenon of absorption or fixation is the result of chemical interchange or due to physical or other causes, there would be a consumption of calcium, magnesium or other base by the sulfate radicle. Since ammonium sulfate is regarded as a physiologically acid salt, in field soils the possible utilization of ammoniacal nitrogen by growing plants would be a cause contributing further to the depletion of soil bases.

Whether the ammonia is changed from its combination as sulfate directly to nitrous acid and nitrate, or whether there is an intermediate reaction, a base will be required, as is evidenced by the results for ammoniacal and nitrate nitrogen in the soils where sulfur was oxidized and variations in basicity existed.

In this connection, two questions are suggested. To what extent will tricalcium phosphate serve as a source of basic calcium in soil which, while not supplied with calcium carbonate, contains minerals which may be attacked by acidity developed through nitrogen transformations, or by the sulfate radicle of ammonium sulfate? The acid radicle will be a factor regardless of whether ammonia is oxidized by nitrite bacteria, fixed by soil or utilized in part directly by growing plants.

And further, if the soil is supplied with calcium carbonate, will the nitrous acid exercise any selective action and unite with the calcium of tricalcium phosphate in preference to calcium present as carbonate?

Without the data for calcium and phosphorus which will be obtained as a part of the investigation, at present not completed, the nitrogen results must be regarded as indicative only. However, the figures for nitric nitrogen found in these mixtures of the acid silt loam soil where floats were included and either one thousand parts of calcium carbonate or none were added, furnish very slight evidence of the calcium in tricalcium phosphate mixed with the soil at the rate of one thousand parts per million having functioned as a base.

When no change was brought about in basicity by calcium carbonate or in acidity by the oxidation of added sulfur, a part of the proteid nitrogen furnished by casein has been completely changed to the nitrate form and a part to ammonia only. The ammonia found was considerably less than was present in mixtures which were similar except that added sulfur was oxidized, and the nitrates were half the amount produced when the base requirement of the soil was satisfied.

SUMMARY

Oxidation of elementary sulfur in soils devoid of base depresses activities of nitrifying organism.

Although basicity was supplied by calcium carbonate in excess of the requirement of the soil, the yield of nitric nitrogen from casein was considerably decreased by oxidation of added sulfur.

A further decided depression occurred with decreased basicity, so that a quantitative relationship exists between the yields of nitrates and calcium carbonate additions.

The increasing amounts of ammonia which accompanied the decreased yields of nitrates when sulfur was oxidized, can not be considered as indicative of sulfofication having exerted a stimulating effect on ammonification.

This inverse relation between ammonia and nitrates must be interpreted as existing rather because the deficiency of base necessary for neutralizing sulfuric acid has inhibited the transition from ammonia to nitric nitrogen. The ammonia formed neutralized the acidity and remained as ammonium sulfate.

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THE IMPORTANCE OF MALARIA TO AGRICULTURE AND STUDIES ON MALARIAL SOIL

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It is well known to all, whether it be in Africa or America, how very often malaria is an obstacle to the permanent occupation of the soil by man. As a matter of fact, there are numerous cases of expeditions of explorers and travelers destroyed, and of attempts at colonization impeded on the "Dark Continent." It is also well known that in the cutting of the Panama Canal the major obstacle was malaria in conjunction with yellow fever.

But even in Europe malaria is still an obstacle to the progress of agriculture. Southern Italy, southern Spain, southern Russia, Greece and nearly all of the Balkan Peninsula are strongly malarial. As to farming in these regions, as the saying is in Italy, internal colonization finds its principal obstacle in malaria. These regions are characterized:

(a) By lack and scarcity of rural population. On the other hand, population is congested in the settled centers. Very often these centers are very distant from each other.

(b) By the predominance of large estates under extensive culture as compared with small estates under intensive culture.

(c) By lack of country houses and roads.

(d) By disorders of stream action (erosion) due to lack of systematic handling of the land, which is manifested by the torrents in the deforested hills with inundations and swamps in the low lands.

(e) Finally, by the prevalence of malaria, which above all else in the summer interferes with the housing of workers in the country.

Besides this group of causes, which in their turn are effects, historians point out remote causes in the large estates of the Roman Empire, in the Saracenic invasions (which compelled the population to take refuge in the hills) in feudalism and in feudal legislations, in brigandage, in the occupations by strangers (enemy or conquerors), in the destruction of forests, etc. These conditions, until a short time ago, formed a closed cycle which nothing could break; extensive culture, the large estate, the swamp-engendered malaria, which in turn gave rise to the depopulation. This depopulation resulted in extensive culture, large estates, pasture feeding, and the abandonment of the soil.

However, the studies of Laveran, Celli, Marchiafava, Ross, Manson and Grassi, had made certain that malaria is propagated exclusively by means of mosquitoes of the genus *Anopheles*. They demonstrated that the protozoan parasite passes a closed evolutionary cycle between the *Anopheles* and man. It was thought for a certain time that by improving the health of the malarial person or curing with a specific remedy, and also through keeping healthy people from contracting the disease from bites of infected *Anopheles* by screening, malaria would be destroyed in a few years. Thus the principal obstacle to the possession of much land would be removed.

It was at this time that the malarial flagellum was considered a conquered giant. The illusion faded very quickly. The curing of malarial patients in the preepidemic period was proved to be too inefficient in diminishing the quantity of malarial virus quickly. Mechanical protection with netting on doors and windows, and with gloves and masks, appeared to be a form of nonsense practical for the most part only as a luxury.

In the meantime, however, a new horizon had formed about hygiene. It was said that, since malaria is carried by the *Anopheles* which has its home in water, removing the water from the malarial places by means of well-conducted improvements, ought to effect the reclamation of the malarial locality by removing the means of malarial propagation.

It had to be recognized very soon that the mere presence of *Anopheles* was not the true criterion of the possible prevalence of malaria. The number of *Anopheles* was not synonymous with the epidemic; that is, it was possible to have entire regions badly infested, with little malaria prevalent, and on the other hand, it was possible to have a severe malarial condition with a ridiculously small number of *Anopheles*. Thus it was discovered that there could be freedom from malaria with swampy conditions and a prevalence of *Anopheles*. It was ascertained that there are zones in all latitudes in which malaria arises, which are notoriously swampy and give rise to a very abundant *Anopheline* fauna, but that malaria in these places is well-nigh only a matter of historic record.

This was enough to demonstrate that the equation

$$Man \times Anopheles = Malaria$$

was incomplete and that instead it should be expressed

$$Man \times Anopheles \times x = Malaria$$

In continuing researches on the aquatic habitat of the *Anopheles*, its enormous facility for adaptation was recognized. It was seen that the phenomenon of *Anophelism* without malaria was the rule and not the exception. Hence the etiological formula, that where the *Anopheles* exists there is malaria, should be changed into the following which the author has proposed: Where there is malaria there is the *Anopheles* which transmits it.

But whatever other disillusions these last proofs did not bring forth, there was a true debacle of every scientific application to practice!

It was thus feared for a while that we should have to renounce every hygienic effect of drainage. It was thought that it was mere nonsense to hope that drainage could be so well conducted as to remove the possibility of propagating *Anopheles* in a territory in which they had been established for centuries. Since the *Anopheles* might not find any more water truly stagnated, they would content themselves with wells, drinking fountains, ponds in gardens, wash tubs and puddles, and in the absence of fresh waters, would end up in making use of salt waters.

It would have been a case of despairing of every progress based on the new discoveries, if two new proofs had not come to our aid. These can be summarized thus:

(a) It is possible to live in malarial places by means of modern prophylaxis, both general and quininic.

(b) Drainage improvement, which has behind it agrcultural improvement, that is, intensive cultivation of soil already malarial, makes the malarial regions sanitary again.

In the first point there is a plebiscite of scientists and conscientious experimenters. As to numerical examples, they do not stand in any need. Those reported in the table of Celli were published in the paper on malaria, entitled "Work of the Society for the Study of Malaria from 1898 to 1908." On the second point, there is also a plebiscite determined by a series of objective and systematic studies.

In fact, from studying the major part of Italian improvement (drainage), it has been concluded that where for particular reasons it has been possible to establish agricultural improvement, immediately after drainage improvement there has also been a hygienic improvement. The contrary resulted where it was not possible to have agricultural improvement as a complement to the drainage.

The author's station has carried no light contribution of studies in this sense. Among these may be recorded:

(a) Studies on the improvement of the province of Reggio Emilia (northern Italy) which have made the fact prominent that the said province can be divided into two regions: a low region with much surface waters, with a great prevalence of *Anopheles* and a small amount of malaria; and a high region without surface waters worthy of note, with a prevalence of *Anopheles* sufficiently serious, yet in which it may be claimed that malaria has disappeared. The only historic difference between the two regions lies in the fact that in the high part of the Reggian Plain, drainage improvement is antiquated while on the low region it is relatively recent.

(b) Observations made about the province of Macerata (one of the five provinces of Italy immune from malaria for some time and under intensive agriculture), which led to establishment of the fact that while malaria had not existed at the place for about one century past, with the last drainage improve-

ment and in the latest rice fields, the prevalence of *Anopheles* is still very great and has an influence upon homes near the city.

(c) Studies and researches upon the celebrated ex-Lake Fucino, drained by the House of Torlona. These show that if in the Fucine Region the swamp, has always existed, and hence presumably the *Anopheles* also, malaria there has never become intensified. There was an exception to this in a period of a few years which coincided with the work of drainage and which gradually disappeared while agricultural improvement was becoming established on the drained banks.

(d) Observations and verifications in which we are assisting in many localities of the south. If in itself malaria has very often impeded the advance of agricultural improvement in its intensity, the fruitfulness of the climate has also permitted of other means of resanitation following drainage and agricultural improvement. This has taken place in lands of pronounced malarial conditions. It is noteworthy from this viewpoint, in the negative sense, as regards portions of Vico in the drainage of the smaller basin of the Volturno, until almost up to the reign of Ferdinadn II.

The Bourbonic administration had well understood that to complete the gigantic works accomplished, it was necessary to add private to the public improvement. In addition to this, in so far as the administration was able, it had allotted to the inhabitants of Vico di Pantano a good portion of the reclaimed land, constructing for the most part a small house for each portion. The air was still "mefitic," to use the language of the time. This did not permit the peasants to sojourn in the open country. The houses were abandoned (the remains may still be seen) and the portions left were reformed into the large estate.

The Colony of Battipaglia, constructed almost contemporaneously as a place for fugitives from the earthquake of Melfi, also failed in its scope. There was even a greater failure also about the plain of Eboli, as every inhabited center made its influence felt for a considerable radius about itself. On the other hand, the gardens of Schito and all the valley of the Sarno succeeded in becoming sanitary again in a little less than half a century. Here the dominant factor was the most intensive of culture, as is followed in turning the soil four times annually in cultivating kitchen gardens. It is all the more worth while to consider the swamps of Naples, from which malaria has disappeared (or nearly so) since each zone could be cultivated.

Nor is there a lack of very recent examples. Let the reclamation of the estate of Pantanaat Castelnuovo Vallo suffice for all. Here the disappearance of the epidemic has kept pace with an increase in farming and with the breaking up of the large estate, thanks to quininic prophylaxis.

(e) Studies and observations (not yet edited) made on the swamps in the provinces of Pisa, Livorno and Lucca, which are almost entirely without malaria. Studies on Massarosa, Massachiucoli and the vicinity of Lucca, etc., have shown that here also the only explanation of the fact is that not one

square meter of soil not submerged is left uncultivated. Even the water, so to speak, is cultivated, being used in the rice fields.

(f) Studies and observations (not yet edited) made in France, in the vicinity of Paris, Sarthe (Le Mans, Bonnetable), and Normandy (Alengon, Le Ferté Bernardete) which have confirmed that these regions, in which malaria is not even more than a matter of historic record, abound in swamps and in *Anopheles*. However, they are under intensive agriculture in all regards.

In other words:

(a) A positive connection certainly exists between intensive cultivation of the soil and a diminution and even a disappearance of malaria. This is not necessarily in conjunction with a decrease in surface waters, capable of harboring *Anopheles* larvæ.

(b) Neither the numerical gravity of malaria (or the coefficient of morbidity) nor the gravity of the infections (or the coefficient of perniciousness) is as a matter of fact in relation to the number of *Anopheles* of a certain territory.

(c) It has been impossible as yet to find any difference between the innocuous *Anopheles* of a reclaimed zone and the harmful *Anopheles* of a pestiferous zone.

(d) In the major number of cases it has been and is entirely possible to see that the spontaneous retrocession of malaria has been contemporaneous with the advance in the intensification of culture (or of the resumption of farming operations). This intensification was not necessarily for the entire territory so as to give the idea that it was due to a decrease in surface water. It was done on the parts free from water and therefore susceptible to culture. From all this the only conclusion in keeping with logic is that the remanagement of the soil has an unfavorable influence on the cycle *mosquito—man—mosquito* to the point of breaking its implacable continuity. This can be practically expressed by saying that the *Anopheles* of the cultivated zones become incapable of transmitting malaria. This influence must be specific even in spite of the fact that rare exceptions to this rule have been indicated. This may mean that the specific condition is not always present.

It may also be that such a condition may exist as the presence of a germ in the ground in a stage unknown to us, and which is killed by conditions of environment created by cultivation. We cannot claim to know all that may be known some day on the nature of protozoa. We should be constantly on our guard against asserting that we have added the pillars of Hercules to knowledge in any field of biology. From what we continually apprehend from the best-known germs (or those we believe to be best known) nothing authorizes us to admit it, because many other hypotheses can be made which have all the more reason to be, but are invalid, which are equal to the first from every basis of experimentation or observation.

It is very true that with this hypothesis of the malarial germ in the soil, the autoctonal cases of malaria (occurring in 8-day periods) also would be explained, which not even A. Celli denies. Even the hypothesis of a hyperparasite which was favored by the cultivation of the soil and which ended with

the destruction of the amatozoan in the *Anopheles*, the hypothesis of a relative immunity (absolute immunity is excluded) introduced into the *Anopheles* with the change of vegetative nutriment offered them by cultivation (analogous to the softening effect on plants from fertilization and cultural practices) and many other hypotheses have an equal right to be offered. Each of these is as worthy of consideration as any of the others; and who knows that they are not all true and that they are not explanations, for the present more or less plausible, of the fact which no one can deny, namely, that if the soil is put under management again, malarial epidemy becomes attenuated and disappears?

There is a tendency to reduce every thing to formula. The equation

$$Malarial\ Man \times Anopheles = Malarial\ Epidemic$$

as has been said, no longer stands. The other by A. Celli

$$Malarial\ Man \times Anopheles \times xyz = Malarial\ Epidemic$$

is nothing but the expression of our ignorance, because no one has given us the value of the physical, biological and social factors which are represented by the unknown *xyz*. The formula proposed by the author: *Where there is malaria there is the Anopheles that transmits it*, is also destined to restate the bearings of the old experience which people had with malaria, a disease essentially autoctonal and allied with the soil. This may bear relation to the true, perhaps indestructible, bearings of modern science, according to which malarial epidemy is maintained in man only with the bite of the *Anopheles*.

Moreover, science must explain exhaustively the mysterious connection which passes between soil and malaria, or find the true connecting links between the exclusively and closely parasitarian theory and the telluric theory of Tommaso Crudeli.

It is convenient to observe that, seduced by the bewildering exclusiveness of the doctrine of the malarial mosquito, we have disguised the studies of the soil as a malarigenic factor. It is still necessary to turn to the soil, which in its bosom holds the waters that harbor the *Anopheles* larvæ. This soil in conjunction with the waters, forms the mud which often harbors and permits the eggs to hatch. Plain looking waters sustain the aquatic plants of the most various small forms. It has a part in determining the plankton of swampy waters and is always connected with the micro- and macro-fauna, with which *Anopheline* generations come continually into biological conflict. In brief, it permits the natural and artificial development of the plants from which 90 per cent of the *Anopheles*, which are essentially phytophagous, get their nutriment.

Still no fact is available to furnish conclusive proof. The culture of malarial parasites is yet to come, notwithstanding the attempts of Bass and Johns. The study of swamp plants has possibly brought in only a ray of light. Celso Ulpiani's report on the relation between colloids of the soil and malaria has introduced a pleasing intimation.

He says:

The formation of swamps is, according to me, a colloidal phenomenon, as is also malaria, which in our warm climates is always found in conjunction with the existence of swamps. This includes a colloidal problem. I do not know what connection may exist between malaria and swamps, but a connection must exist. The biological cycle of the fatal plasmodium must in some manner be connected with the permanence of the hydrozoa of the soil and with the high temperatures of the climate. Colloidal chemistry will certainly give the explanation for the indissoluble relation which exists among swamp malaria and warm climate. When floods occur in the bottom of a valley in our climates and the assiduous and tenacious farmer does not break up the colloidal deposits resulting left by floods with the plow, the spade and hoe, it favors the agglomeration of these colloids. If for years and years agriculture becomes absent, the colloids deposited on the washed and leached soil begin to obstruct the pores of the subsoil. They glue the particles together while with the diminished access of air the organic matter becomes humified. This protects the organic colloids more and more from coagulation. Upon this soil, which, so to speak, may be said to be "varnished," and which no longer takes up the water, the swamp is found with its black muddy waters. Its mud is slippery and insidious and its flora useless. It has its mosquitoes, its wandering lean animals and a sparse human population degraded by fever.

This is purely a pleasant conception, but one which deserves to be taken into serious consideration by studious people.

Hence the author believes that the courses to be followed by the studious must be new and diverse in the study of the problem. It is true that problems of this nature are hardly solved in a direct way. For the most part, their initial solution (or the first clue) is in accordance with the case. But it is also true that the case generally helps those who place themselves in a position to be helped by it. It is therefore necessary to study malarial soil in all its manifestations in contrast to the now malarial soil, using both fundamental systems of scientific study—observation and the experiment. Observation should include retrospective observation, that is, the history of the soil, either geological history or that concerned with its recent vicissitudes, in other words, the history of its agriculture. It is true that in recent times there has been an outcropping of historical studies on malaria, to which A. Celli has given impetus. For years he collected precious material for the historic study of Roman malaria. He also gave an accurate and perhaps complete bibliography.

The studies which we owe to Ronald Ross, in collaboration with C. C. Ellett, are entitled "Malaria, a Neglected Factor in the History of Greece and Rome." There is also the one by Paul Roux, "The Agricultural Question in Italy." Studies by Dr. E. Genovese on Calabrian malaria, "The Roman Estate," are also worthy of note, and perhaps those made by this station concerning the places improved, which have been reviewed, are not to be neglected. Even in other times the historic study of malaria was overshadowed. Memoirs contained in the first and only volume on the "Annals of Improvements," published through the care of John Savarese, are entitled to honor. The same may be said above all of that by the Canon, John Scherillo, entitled "On the Air of Baia at the Time of the Romans."

But chiefly, the most neglected reports in these studies are of course those interceding between malaria and agriculture, not only that which reflects

"rural economy," but also, and above all, "agricultural technique." The study of the latter is notoriously difficult and actually always incomplete. If, as a matter of fact, we know little about Roman agriculture, notwithstanding the works of Cato, Varro, Virgil, Pliny, Columella, etc., we know less about Grecian agriculture. We know almost nothing about Mediæval agriculture, wherein ever the sources to draw on are lacking. Books and the history of modern agriculture also are lacking.

And yet it may be absolutely necessary to be familiar with this history as to "agricultural ground and malaria" in order to obtain information therefrom in our day on the manner of conducting agricultural improvements. It tells us how much to expect and to count on with regard to the above in order to arrive at the definitive hygienic resanitation against malarial plagues. As to experimentation, it can be and should be carried out in various ways, and, moreover, with more than the ordinary laboratory researches; more properly,

(a) By setting up experiments *ad hoc* in which it shall be possible to reproduce the natural conditions exactly.

(b) With experimental demonstration camps true and proper.

(c) With adequate collections, in which the studious person with his talent shall be able to find as much about the subject as the research of the moment warrants.

The station, under the author's direction, which has been occupied with the study of reports on malaria and agriculture for 17 years, is conscious of the very numerous reports which exist but are not known in the field just mentioned. It is conscious of the difficulties and the relative necessity of their solution. It believed it to be well to confront the problems directly, studying also the swamp plants, the plankton of the swamps, the reports of the *Edaphon* with the problem on malarial soils in connection with the colloidal state and the prevalence of malaria in the territory. For this purpose a collection of material has been created necessary according to the consideration which we have mentioned. In fact, the station has a number of enactments for the production of artificial swamps more or less extensive, ground held under open pasture and pastures with rhyzomacious plants, ponds with water-proof bottoms, both elevated and sunken, also ponds with water-proof sides and porous bottoms, an experimental aquarium, etc. In addition to this, it is providing itself with special equipment without forgetting the proper collections, and above all, collections of soil from swampy and malarial places. It is providing itself with special products of the swamps, a small collection but very interesting, and destined to complete the studies on the *Edaphon* and the plankton in conjunction with malaria. It is the author's intention to publish a catalog which is now in the course of compilation. This is for the use of persons making studies in this field, with the purpose of increasing collections by means of exchanges and communications.

All the collections mentioned are catalogued. The author hopes that at the end of the war he may be permitted to place his equipment and collection at the

disposal of investigators who may wish to collaborate with him for the extinction of one of the most burning plagues of all humanity, the dreaded malarial infection.

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